finalreport

Project code: B.PAS.0312

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Date published: November 2011

ISBN: 9781741917000

PUBLISHED BY

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Reliable Establishment of Non-Traditional Perennial Pasture Species

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

This project was undertaken to develop a suite of reliable, robust and economical establishment packages for the most promising saltland, native and exotic perennial species for recharge, discharge and pastoral areas of southern Australia and to identify low-cost management options for successful recruitment of perennial grass seedlings in native grass pastures.

A mature package for reliable establishment of sub-tropical perennial grasses in south-western Australia has been developed. Adoption has been rapid, with typical establishment densities in commercial paddocks having increased ten-fold. This has contributed to an increase in the estimated area sown to sub-tropical grasses from 120,000 ha to 150,000 ha since the project commenced. A direct seeding package for old man saltbush and *Rhagodia preisii*, suitable for use with conventional farm seeding equipment, has also been developed. No major barriers to germination and establishment were found in the perennial legume, tedera (*Bituminaria bituminosa* var. *albo-marginata*). The project has also identified low-cost management options for successful recruitment of perennial native grass seedlings to increase productivity of native grass pastures. A greater understanding of the seed biology and agronomic requirements has been achieved for other halophytic shrub and native pasture species for future development into reliable establishment packages.

Five technical bulletins, two extension bulletins, three advisory notes and nine scientific papers have either been published or prepared for publication. These provide details of the establishment and seedling recruitment packages, and the scientific basis for the recommendations behind them.

Executive Summary

Background

A range of perennial pasture options have recently been developed and promoted across southern Australia. While the steps for reliable establishment of traditional temperate pasture species from seed are well understood, this has not been true for non-traditional species, notably halophytic shrubs (particularly saltbushes), native pastures and exotic sub-tropical perennial grasses. Very little is also known about the germination requirements of tedera (*Bituminaria bituminosa* var. *albo-marginata*), which is currently being researched as a new perennial pasture legume. The constraints and risks associated with establishment of these pasture options need to be overcome, in order to increase their adoption and realise their productivity and environmental benefits. Pastoral zone graziers also need low-cost techniques to rehabilitate large areas with saltbush.

The grazing industries in high rainfall, non-arable areas of eastern Australia are reliant on native pastures for production. Many of these have become degraded and improved management strategies are required to increase the density of desirable native grasses to maximise productivity. One means is to enhance natural recruitment from seed. But in order to do this, a greater understanding of the seed biology and germination requirements of native grasses is required.

This project was undertaken to develop a suite of reliable, robust and economical establishment packages for the most promising saltland, native and exotic perennial species for recharge, discharge and pastoral areas of southern Australia and to identify low-cost management options for successful recruitment of perennial grass seedlings in native grass pastures. The native grass establishment component was conducted in conjunction with the RIRDC-funded project "*Grass roots – native perennial grasses for sustainable pasture systems*", while the native grass recruitment component was conducted to enhance the MLA-funded project "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*".

Achievements

The project was successful in developing establishment packages and an increased understanding of the seed biology of key species in each of the target groups. Five technical bulletins, two extension bulletins and three advisory notes that provide details of the establishment and seedling recruitment packages, and the scientific basis for the recommendations behind them, have been prepared for publication. Six scientific papers have been published or submitted to the journal *Crop and Pasture Science*, while three other papers are in preparation.

1. Exotic sub-tropical perennial grass establishment

A mature package for reliable establishment of sub-tropical perennial grasses, particularly Rhodes grass (*Chloris gayana*), panic grass (*Megathyrsus maximum*), signal grass (*Urochloa brachiaria*), setaria (*Setaria spp.*), digit grass (*Digitaria eriantha*), Bambatsi panic (*Panicum coloratum*) and kikuyu (*Pennisetum clandestinum*), has been developed. At the commencement of the project, seeding failures were high and establishment was often patchy with a mean establishment density in the order of 1 plant/m^2. Less than four years on, establishment densities of 20-50 plant/m^2 have consistently been achieved. Close interaction with the Evergreen group, catchment councils and seeding contractors has led to rapid adoption of the technology, with subsequent increases in establishment success throughout the industry; there is now an expectation by farmers of achieving establishment densities of > 10 plants/m^2.

2. Saltbush establishment

The project developed a technique for direct seeding old man saltbush (*Atriplex nummularia*) with conventional farm machinery, thus allowing extensive plantings of this species at low cost. Precise sowing depth of 5-10 mm is critical for success. Subspecies *spathulata* was found to be unsuited to direct sowing, while genotypic differences were found within ssp. *nummularia* for ability to establish from seed; seed of these types have been included in the FFI CRC saltbush breeding program. The technique was also successful for mallee saltbush (*Rhagodia presseii*), but further work is needed to develop direct seeding methods for other saltland species.

3. Native perennial grass establishment

The use of dormancy breaking treatments and germination stimulants, such as smoke water, gibberellic acid, heat treatment and manipulation of florets, improved germination in the laboratory of several native grass species from 5% to >90% in some cases. One or more of these treatments improved germination of *Austrodanthonia* species, *Chloris truncata*, *Themeda triandra*, *Dichanthium sericeum*, *Enteropogon acicularis* and *Microlaena stipoides*. Species differences were also found for after-ripening and storage treatments. The translation of these treatments to establishment in the field requires further research.

4. Tedera (*Bituminosa bituminaria* ssp. *albo-marginata*) establishment

Tedera is a promising new drought-tolerant perennial legume from the Canary Islands being extensively researched by the Future Farm Industries CRC. However, little is known of its germination characteristics. A study, attached to this project, by an Honours student from Curtin University showed no major barriers to its establishment in the field. It is well suited to sowing in autumn-early winter and will emerge from a sowing depth of up to 10 cm. An establishment package has been developed for the species, in readiness for its commercialisation.

5. Recruitment of native grasses

Low-cost, ecologically-based management options enabling the successful recruitment of perennial grass seedlings within existing pastures were identified for the native grasses *Austrodanthonia* spp. and *Bothriochloa macra*. Seedling recruitment was greater when seed set was higher, more herbage mass remained in intact swards, some soil disturbance was implemented at seed maturity, insecticides controlled seed-harvesting ants and herbicide application after seed maturity controlled weeds. The surface soil needs to be moist for >7 days in late summer- early autumn for high seedling densities. Rainfall records indicate that useful recruitment should occur in most years.

6. Seed quality

Poor seed quality was identified as a major factor limiting establishment success in all perennial species studied. An education program to make growers more aware of seed quality issues is likely to have the greatest effect on influencing the seed industry to increase seed quality standards.

Industry benefits

The establishment packages developed in this project will benefit graziers by enabling them and seeding contractors to establish a range of saltland, native and exotic warm season perennial pasture species from seed with low risk and at an affordable cost. Farmers will benefit directly from increased farm productivity and provision of out-of-season feed provided by perennials. Widespread adoption of the packages will benefit the community through increased planting of deep-rooted perennials that help reduce rising water-tables and the impacts of dryland salinity. This project, in conjunction with the MLA-funded PhD project, has also developed a management package for graziers in eastern Australia to increase pasture productivity through enhanced recruitment of desirable native perennial grasses, at a significantly lower-cost than re-seeding with exotic species.

Adoption of the establishment package has been rapid for the sub-tropical perennial grasses and there is now an expectation of achieving establishment densities of > 10 plants/m^2, compared to less than 1 plant/m^2 when the project commenced. The estimated area sown to sub-tropical grasses in WA has increased from 120,000 ha to 150,000 ha since the commencement of the project. Greater confidence in achieving establishment success has been a key driver in increased adoption.

The experimental package for old man saltbush has been transferred to a group of leading farmers and seeding contractors, who are keen to develop this concept further for sowing large areas with their own machinery. An establishment package has been developed for tedera, in readiness for its commercialisation. A greater understanding of the seed biology of native grasses has led to increases in their germination success, but further work is needed to translate this into reliable establishment packages in the field. In eastern Australia, graziers now have reliable, low-cost strategies available to them to increase native grass content in existing native pastures.

Contents

1 Background

A range of perennial pasture options have recently been developed and promoted across southern Australia. While the steps for reliable establishment of traditional temperate pasture species from seed are well understood, this has not been true for non-traditional species, notably halophytic shrubs (particularly saltbushes), native pastures and exotic, sub-tropical grasses. The constraints and risks associated with establishment of these pasture options need to be overcome, in order to increase their adoption and realise their productivity and environmental benefits. Pastoral zone graziers also need low-cost techniques to rehabilitate large areas with saltbush.

1.1 Chenopods for saltland and rangeland environments

Saltland is a difficult environment for plant establishment and growth. Our understanding of the germination and establishment processes in these hostile soils is rudimentary. Farmers seeking to establish halophytic shrubs on saltland can either: (a) seed directly using the niche seeding technique developed by C.V. Malcolm and colleagues in the 1970s (Malcolm and Allen, 1981), which deposits *Atriplex* fruits mixed with a vermiculite mulch at 1-3 m intervals along a raised M-shaped bank; or (b) they can use commercial tree planters to plant nursery-raised seedlings. The use of nursery-raised seedlings is generally more reliable than niche seeding (Barrett-Lennard *et al*. 1991). However, direct seeding (~\$100–150/ha) is far cheaper than the planting of nursery-raised seedlings (~\$450/ha). Farmers, therefore, face a dilemma between risk and price: the establishment technique that is most reliable is too expensive to implement; while the less expensive technique is too risky. Furthermore, the cost of sowing these species for rehabilitation of large pastoral zone areas is currently prohibitive and too risky. If prices for *demonstrably reliable* saltland and rangeland pasture establishment could be brought down to ~\$120/ha, there would be substantial farmer and grazier adoption. Furthermore, there is a high chance that the developed techniques would be appropriate to the adoption of direct seeding for applications in the rangeland and arid areas of the wheatbelt.

There are two approaches to developing direct seeding techniques for more reliable chenopod shrub establishment. One is to better engineer the sowing niche. Maximum growth of shrubs is possible with stands of ~1,000 stems per hectare (Malcolm *et al*. 1988; Barrett-Lennard 1993). Thus, an outlay of \$100–\$120 per ha for establishment equates to a cost of 10–12 cents per seed placement. This allows for the possibility of relatively expensive micro-engineering options to help overcome a number of stresses affecting establishment including salinity, waterlogging, inundation, drought, weeds, insects, grazing, and low temperatures/frost (Malcolm, 1972; Jennings et al. 1993; Barrett-Lennard *et al*. 2003). The alternative viewpoint is to develop direct seeding methods in which farmers can use their own seeding equipment. This project elected to follow this approach, with the main target area being those more favourable to chenopod growth - mildly to moderately saline areas, not subjected to prolonged waterlogging.

Relatively little is known about the seed germination requirements of many of the main chenopod shrub species of interest. A greater understanding of their seed biology and the environmental factors that trigger germination is needed, before more reliable establishment methods can be developed. Such issues include physical or embryo dormancy, the role of bracteoles enclosing the seed, and optimal temperature and moisture conditions for germination. Given the harsh micro-environment in which these species grow, often characterised by high salinity and drought, the development of seed treatments that maximise the potential of the seed to germinate and emerge rapidly could significantly enhance establishment success (Powell 1998).

In a pilot project DAFWA and Kings Park identified several seed treatments in the laboratory that had a significant impact on germination. Most work was conducted on the three major saltbush (*Atriplex*) species sown commercially – old man saltbush (*A. nummularia*), river saltbush (*A. amnicola*) and wavy leaf saltbush (*A. undulata*). Some work was also conducted with creeping saltbush (*A. semibaccata*) and silver saltbush (*A. bunburyana*). These related to bract removal, light treatment and chemical priming (pre-germinating) of seeds with water or germination stimulants, such as the plant signalling chemicals karrikinolide (the active ingredient in smoke) (Flematti *et al.,* 2004), gibberellic acid, benzoic acid (Senaratna *et al.*, 2003), salicylic acid (Senaratna *et al*., 2000) and cytokinins.

This project aimed at testing the preliminary results for these species in the field, in combination with gaining a greater understanding of their seed biology and agronomic requirements for germination and emergence, in order to develop reliable, low-cost direct seeding options. In particular, the use of conventional farm machinery for sowing was examined, to encourage farmers to sow their own seed.

1.2 Sub-tropical perennial grasses

Sub-tropical perennial grasses in Australia have traditionally been grown on the northern slopes and plains of New South Wales and in Queensland. However, they are now also being widely used in both the Northern Agricultural Region (NAR) and south coast areas of Western Australia, particularly on waterlogged or deep leached sands that are uneconomic to crop. Kikuyu (*Pennisetum clandestinum*) has been grown on the south coast and Swan Coastal Plain for many years, while sowings of Rhodes grass (*Chloris gayana*), panic grass (*Megaththyrsus maximus* syn. *Panicum maximum*), signal grass (*Urchloa brachiaria*), setaria (*Setaria sphacelata* and *S. splendida*), and to a lesser extent digit grass (*Digitaria eriantha*) and bambatsi panic (*Panicum coloratum*), have increased in the past 10-15 years (Moore *et al.* 2006). This has largely been driven by farmer groups, such as Evergreen.

Productivity benefits from use of sub-tropical grasses include increased carrying capacity, due to provision of out of season green feed, improved seasonal distribution of feed and reduced reliance on supplementary feed and conserved fodder in autumn, while environmental benefits include increase water use and reduced groundwater recharge (with reductions in the potential for dryland salinity) and reduced wind erosion potential in summer (Moore *et al.* 2006). Sub-tropical grasses can be grown on a wide range of soils, although their main use in Western Australia has been on sands that are marginal or unsuitable for cropping and to coastal areas subject to periodic waterlogging. On these soils, well managed perennial-based pastures can significantly increase production compared with annual volunteer pastures, in addition to their environmental benefits.

While some of the factors for successful establishment have been developed for northern New South Wales, establishment is more problematic in the Mediterranean-type climate of south-western Australia. In northern New South Wales sub-tropical grasses are best sown in November-December, when soil temperatures are warm and there is a strong likelihood of summer rainfall, and can even be successfully sown as late as January (Lodge and Harden 2009). In south-western Australia the optimum sowing window in spring is narrow, due to the need for warm soil temperatures for germination conflicting with decreasing rainfall reliability. This results in plants trying to establish in drying soil profiles. A further challenge is the sand-based texture of the majority of soils in the Western Australian target zones, which are typically water repellent and prone to rapid drying in spring.

Seeding failures of sub-tropical grasses have been common in south-western Australia, with establishment densities often less than 1 plant/m^2 or a mixture of good and poor patches across the paddock. This low plant density is a major impediment to the potential production from these sub-tropical grass pastures, especially for the bunch grasses (panic grass, digit grass, setari and Bambatsi panic grass), which have negligible ability to recruit new seedlings. However, the regular success by a small group of farmers and promising results in experimental trials indicated that successful establishment of sub-tropical perennial grasses was possible.

The conditions for successful establishment of sub-tropical perennial grasses have not been well understood by farmers nor been well underpinned by science. On the one hand, unless the constraints and risks associated with establishment of these pasture species can be overcome, their adoption will decline and their productivity and environmental benefits will not be fully realised. However, if a reliable establishment package can be developed, the rate of adoption could dramatically increase. The challenge is to develop a robust and reliable establishment package that gives consistently good results. Higher establishment densities will also allow a significant reduction in seeding rates, reducing establishment costs and increasing adoption.

This project was undertaken to gain a greater understanding of the seed biology and agronomic requirements for germination and establishment of sub-tropical perennial grasses, in order to develop a reliable establishment package for farmers and seeding contractors in south-western Australia.

1.3 Establishment of native grasses

Native perennial grasses have a dual role to play in southern Australia. From an agricultural perspective, they are well adapted to local environmental conditions and can provide nutritious feed for livestock. They can also be used for rehabilitation of degraded land and to redress the loss of plant biodiversity. Currently, low availability of viable seed and poor seedling establishment commonly results in unsuccessful native perennial pasture production. By alleviating seed-based issues, pasture productivity will increase and land restoration with native grasses will become more attractive.

The objective of this sub-project was to address these problems though an investigation of the best means to achieve a high establishment success by alleviating after-ripening, developing mechanisms to break seed dormancy, using seed coatings and seed priming and by developing a greater understanding of seed quality issues (viability, germination rates and germination percentage).

Much of this work was conducted in the RIRDC-funded project *Grass roots – native perennial grasses for sustainable pasture systems* in collaboration with Ian Chivers of Native Seeds Pty Ltd. This sub-program provided a link to field-based activities to investigate the effect of these treatments.

1.4 Recruitment of native grasses in native pastures

The grazing industries in high rainfall, non-arable areas of south-eastern Australia have traditionally been reliant on native pastures for animal production. Kemp and Dowling (2000) suggest a paddock composition of at least 60% of desirable native grasses is needed to optimise pasture productivity and sustainability. Many pastures, however, have become degraded, with grass populations below sustainability levels. Kemp and Dowling (2000) found the current perennial content of many pasture systems is often ≤ 20% of pasture composition, well below the desired level. The loss of perennial grasses and their replacement by annual species has had severe implications for the productivity of livestock enterprises by increasing the risks of weed invasion, erosion, salinity and acidity and reducing biodiversity (Kemp *et al*., 2000; Michalk *et al*., 2003). Sustainability of these grasslands is of major concern to landholders and the community at large. A majority of farmers surveyed in the high rainfall temperate zones of Australia also believed it was worthwhile re-sowing old or degraded pastures, even though they may not always make an initial profit from them (Reeve *et al*., 2000). This is indicative of a productivity decline and the desire of farmers to restore paddocks to previous productivity levels.

Improving the perennial grass content of pastures has previously been attempted by sowing exotic species, but in many areas this is difficult, due to steep terrain, or is unprofitable. The average cost of $300/ha generally means 8-10 years are needed to recover costs (Vere *et al*., 1997; Bolger and Garden, 1998), which is particularly problematic on soils of low fertility and in low rainfall areas. Re-sowing also results in a short term loss of production while the newly sown pasture grows. With declining terms-of-trade for livestock production and increasing climate variability and its associated risks, the relative cost of re-sowing has become more marginal, except on more fertile soils and in higher rainfall environments where the chances of success are greater.

An alternative, lower cost method of pasture renovation is to manage perennial pastures in a way that allows successful recruitment of new plants of desirable species. This requires development of low-cost, ecologically-based management practices for existing pastures that contain desirable perennial grass species, by enabling the recruitment of seedlings from seed set by the existing plants. In this way perennial grass content can reach desired levels over time. Using such an approach is likely to be less costly and risky than re-sowing, particularly on less fertile soils and in low rainfall areas. Many degraded pastures still have a residual level of desirable perennial grasses that could be used as the basis for seed production and recruitment of new plants, provided methods to manage the process can be devised. Such procedures need to target: (i) the successful production of viable seeds; (ii) the successful delivery of those seeds to the soil surface for establishment to occur; and (iii) the successful recruitment and survival of young plants.

Recent national research programs have concentrated on increasing perennial grass content using tactical management (Kemp *et al*., 2000, 2003). Much of that work has developed effective management practices that maintain existing plants in a productive state, but has not provided insights into how existing plants are replaced through seedling recruitment (Kemp *et al*., 2000). The latter requires an understanding of the population dynamics of perennial species, which is poorly understood. Pastures based on perennial grasses can be sustained in the short-term through reducing grazing pressure and encouraging vegetative growth, but may fail to recruit new seedlings in the absence of suitable environmental and management conditions. The result is the degeneration of pastures over time.

Understanding how to encourage recruitment of new seedlings into existing swards is a substantial knowledge gap in grassland ecology. Limited research has shown that little, if any, desirable grass seedlings develop into mature plants in existing paddocks, reflecting a poor understanding of the mechanisms underlying the recruitment process. In a study of seed dormancy, germination, seedling emergence and survival of perennial pasture grasses in northern New South Wales, Lodge (2004) posed the challenge of identifying the causes that lead to limitations in successful recruitment. This clearly highlights the need for more research to devise appropriate and practical management options to encourage the emergence and survival of new seedlings into pastures. The work of Lenz and Facelli (2005) in South Australia on recruitment of both native and exotic perennial grass further identified the need for research to better understand the reasons behind the low survival of perennial grass seedlings.

This project investigated a three-phase approach to develop simple low-cost management procedures to enhance the content of desirable perennial grass species. Management strategies were designed to encourage recruitment by: (i) encouraging seed set and shedding by desirable species; (ii) preparation of more suitable micro-sites for seedling recruitment; and (iii) identifying the best post-emergence tactics to aid seedling recruitment in the short to medium-term. Research was conducted on the native grasses, red grass (*Bothriochloa macra*), a C4 species, and wallaby grass (*Austrodanthonia* spp.), a C3 species to develop general principles applicable to a range of native grass species. Much of this work was conducted as a PhD project through Charles Sturt University funded by Meat & Livestock Australia, titled "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*" (Thapa 2010).

1.5 Establishment of tedera (additional sub-project)

Tedera (*Bituminaria bituminosa* var. *albomarginata*), is a perennial legume from the Canary Islands, that is undergoing intensive research through the Future Farm Industries Cooperative Research Centre (FFI CRC) as a potential new pasture legume for southern Australia. There is a paucity of research on all aspects of tedera, although it is known to be well adapted to Mediterranean-type climates and can survive in environments that receive as little as 150 mm annual rainfall. However, little is known about the suitability of this species to southern Australian farming systems. Research into its seed biology is important to understand its germination requirements and to determine whether there are likely to be any impediments to its establishment in the field.

An Honours project through Curtin University was conducted to study the seed biology of tedera, to gain a better understanding of its germination requirements and to determine whether there are likely to be any barriers to its establishment in the field. Factors considered included seed quality issues, dormancy and imbibition characteristics, temperature, light and moisture requirements for germination, the effect of priming with plant signalling compounds and determining optimum sowing depth (Beard 2009).

2 Project Objectives

2.1 Aims

The project had four main aims:

1. To provide cost-effective packages for reliable establishment of targeted saltland, native and exotic, warm-season perennial pasture species and tedera for recharge, discharge and pastoral areas of southern Australia;

2. To gain a greater understanding of the seed biology, seed treatments and agronomic practices to enhance establishment capability of these species; and

3. To gain a greater understanding of the seed biology and germination requirements to enhance recruitment of native grasses in native pastures.

4. To provide information to enable development of specialised seeding machinery for cost-effective and reliable establishment of saltland and rangeland species. It was recognised that the eventual development of such machinery would be beyond the scope of this project. However, following early success with old man saltbush using more conventional tillage systems, this initial aim was changed to develop direct seeding technology using conventional farm machinery, to encourage farmers to sow their own seed.

2.2 Planned Outputs

The planned outputs from this project were reliable and practical packages that maximise the chance of successful establishment of saltland, native and exotic, warm-season perennial pasture species and greater recruitment densities of native grasses in native pastures. A range of products was intended for different target audiences.

2.2.1 Establishment packages for saltland, native and exotic, warm-season perennial pasture species

Recipes for cost-effective and reliable establishment techniques were to be developed for targeted saltland, native and warm-season perennial pasture species for recharge, discharge and pastoral areas of southern Australia. Practical management strategies for native pastures that enhance the natural re-establishment of native grasses were also to be developed, in conjunction with the RIRDC-funded project "*Grass roots – native perennial grasses for sustainable pasture systems"* and the MLA-funded project "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*".

The following two extension products were planned as project outputs:

a). A Technical Bulletin, that provides a comprehensive overview of the germination and establishment knowledge of the target species gained in the project (*Target audience*: Extension Officers, NRM Officers, Consultants, Policy Makers, Catchment Management Authorities, Seed Producers, Seed Merchants, Seeding Contractors, Funding Providers, Program Managers, Students); and

b). Practical and farmer-friendly publications that provide specific establishment recipes for individual species and regions, such as Farmnotes/Agnotes/Agfacts series and other extension publications (*Target audience*: Farmers, Pastoralists, Extension Officers, Consultants, Seed Merchants, Seeding Contractors, Catchment Management Authorities).

2.2.2 Scientific publications

At least five scientific papers reporting on laboratory and field results to be published in journals and conference proceedings (*Target audience*: Scientists, Extension Officers, Consultants)

2.2.3 Information for machinery development to establish saltland and rangeland species

Information to enable manufacture of seeding machinery to more reliably establish saltland and rangeland species (*Target audience*: Machinery manufacturers, Seeding contractors).

3 Methodology

3.1 Project management and structure

The project commenced on 1 July 2006 as part of the former CRC for Plant-based Management of Dryland Salinity (Salinity CRC) and its management was continued by the Future Farm Industries CRC, upon its inception in July 2007. Completion date of the project was 30 June 2010.

The project had industry funding from four sources: Meat & Livestock Australia ($320,000), Australian Wool Innovation ($160,000), the former Salinity CRC ($200,000) and Land & Water Australia ($170,000), through the Sustainable Grazing of Saline Lands project. In-kind support was provided by the Department of Agriculture and Food Western Australia (DAFWA), Kings Park and Botanic Garden (Kings Park), Department of Primary Industries Victoria (DPI Vic), Charles Sturt

University (CSU), New South Wales Industry and Investment (NSW I&I) and the University of Western Australia (UWA)

The project comprised the six following interlinked sub-projects (see Figure 1) to combine existing projects with some targeted additional activity:

- Sub-project 1 - Seed biology and enabling technologies;
- Sub-project 2 - Establishment of saltland and rangeland species;
- Sub-project 3 - Establishment of exotic sub-tropical grasses;
- Sub-project 4 - Establishment of sown native grasses;
- Sub-project 5 - Recruitment of native grasses; and
- Sub-project 6 - Establishment of tedera (additional sub-project added)

A field validation module integrated the laboratory and glasshouse research on seed biology and enabling technologies (undertaken in sub-project 1) with wider-scale agronomic research in the other sub-projects. Sub-project leaders provided technical advice to the field validation module and assisted in integration of research findings into wider-scale application.

3.1.1 Project staff

Project Leader	Dr Phillip Nichols (DAFWA)
Research Agronomist (project appointment)	Dr Ronald Yates (DAFWA)
Post-doctoral fellow (project appointment)	Dr Christopher Loo (Kings Park and UWA)
Leader (Sub-project 1)	Dr Kingsley Dixon and Dr Jason Stevens (Kings Park and UWA)
Leader (Sub-project 2)	Dr Ed Barrett-Lennard (DAFWA)
Leader (Sub-project 3)	Mr Geoffrey Moore (DAFWA)
Leader (Sub-project 4)	Ms Meredith Mitchell (DPI Vic)
Leader (Sub-project 5)	Professor David Kemp (CSU)
Leader (Sub-project 6)	Dr Phillip Nichols (DAFWA)
Technical officer (project appointment)	Mr John Titterington (DAFWA)
Technical officer (project appointment)	Mr Bradley Wintle (DAFWA)

Additional scientists linked to the project included:

- Ms Samantha Clarke (Kings Park and UWA), who was the lead researcher on the linked RIRDC-funded project "*Grass roots – native perennial grasses for sustainable pasture systems";*
- Dr Greg Lodge (NSW I&I), who provided linkage to field activities in northern New South Wales through linkage to the Salinity CRC-funded project "*Agronomy of new perennials*", and
- Dr Megan Ryan (UWA), who provided linkages to various student project activities in native perennial species.

Two student projects were conducted in association with this project:

- Mr Roshan Thapa completed his PhD studies through Charles Sturt University on the MLA-funded project "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*" (Thapa 2010); and
- Mr Cale Beard completed an Honours project through Curtin University titled "*Germination ecology of* Bituminaria bituminosa *var.* albo-marginata *and its suitability to the Mediterranean-type climate of Western Australia*" (Beard 2009).

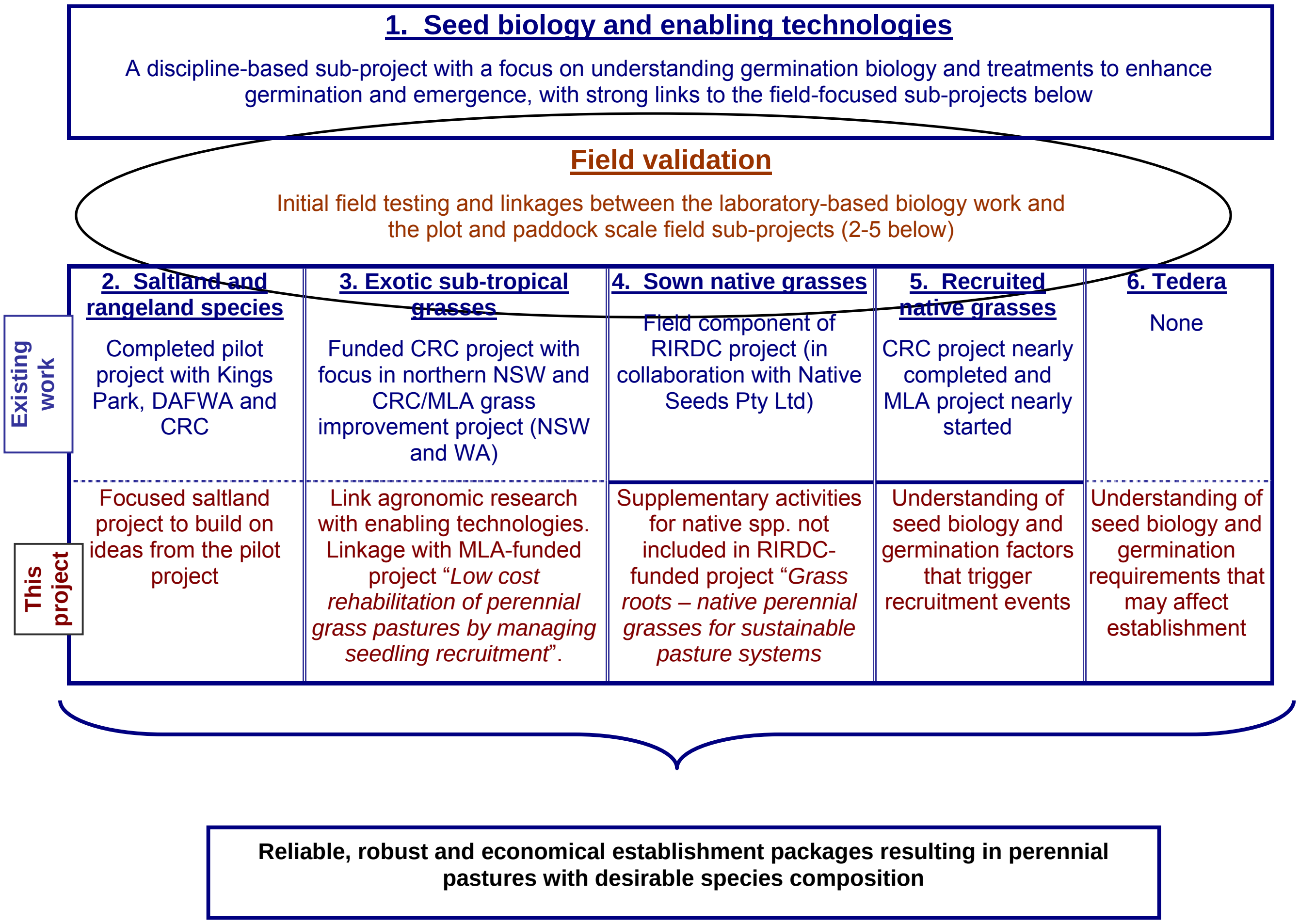

Figure 1 – Diagrammatic representation of the six interlinked sub-projects and the field validation module of the project that combined new activities with existing work at the commencement of the project

3.2 Sub-projects

3.2.1 Sub-Project 1 – Seed biology and enabling technologies

The project provided a post-doctoral research fellow (Dr Christopher Loo), through the University Of Western Australia, to provide a better understanding of the seed biology and germination requirements on priority species and to investigate the potential of seed treatments to enhance germination and emergence. The position was based at the Kings Park Laboratories, which have an international reputation for their work in understanding the biology and dynamics of germination, particularly for native species.

Previous research with some agronomically important species, including saltbush (*Atriplex* spp.) and native grass species (*Microlaena stipoides*), identified several possible dormancy alleviation and germination stimulation strategies with the potential to dramatically enhance germination and seedling establishment. These include chemical manipulation of the seed with germination stimulants including butenolide (the active ingredient in smoke) (Flematti *et al.,* 2004), gibberellic acid, benzoic acid (Senaratna *et al.*, 2003), salicylic acid (Senaratna *et al*., 2000) and cytokinins, priming (pre-germinating) and bract removal (saltbushes). Various seed coatings to facilitate local applications of germination enhancing chemicals, nutrients, fungicides and insecticides and to increase flowability in seeding equipment are also under development.

The activities of this sub-project expanded on previous studies to include further saltland species, and commence new studies of the exotic warm season perennials and other native species to gain a fundamental understanding of their seed and germination biology and to investigate the potential of some new technologies (seed treatments, priming, coatings etc.) to enhance their establishment success. These outputs were then validated in the field validation module for development of practical field-safe establishment options.

3.2.2 Sub-Project 2 – Saltland and rangeland species

This sub-project aimed to build on the results of a pilot project conducted by DAFWA and Kings Park, which identified several seed treatments in the laboratory with a significant impact on germination. Previous work was mainly conducted on old man saltbush (*A. nummularia*), river saltbush (*A. amnicola*) and wavy leaf saltbush (*A. undulata*), with some work on creeping saltbush (*A. semibaccata*) and silver saltbush (*A. bunburyana*). These related to bract removal, light treatment and chemical priming (pre-germinating) of seeds with water or germination stimulants, such as the plant signalling chemicals karrikinolide (the active ingredient in smoke) (Flematti *et al.,* 2004), gibberellic acid, benzoic acid (Senaratna *et al.*, 2003), salicylic acid (Senaratna *et al*., 2000) and cytokinins.

This sub-project aimed at developing a greater understanding of the seed biology and agronomic requirements for germination and emergence of priority saltland and rangeland species. Preliminary results were then tested for their effectiveness in the field in the field validation module. Agronomic studies to develop more reliable, low-cost direct seeding methods were also conducted in the field validation module. The initial aim was to provide information to enable development of specialised seeding machinery for cost-effective and reliable establishment of saltland and rangeland species. However, following early success with old man saltbush using more conventional tillage systems, this aim was changed to develop direct seeding technology using conventional farm machinery, to encourage farmers to sow their own seed.

3.2.3 Sub-Project 3 – Exotic sub-tropical perennial grasses

This sub-project aimed to develop an establishment package to improve the reliability of sub-tropical perennial grasses in south-western Australia, which are attracting increasing interest by farmers for their potential out-of-season productivity, reduction in soil erosion risk over summer and their ability to reduce groundwater recharge. While some of the factors for successful establishment have been developed for northern New South Wales, establishment is more problematic in the Mediterranean climate of south-western Australia, where the optimum sowing window in spring is narrow, due to the need for warm soil temperatures for germination conflicting with decreasing reliability of rainfall after sowing. A further challenge is the sand-based texture of the majority of soils in the WA target zones, which are typically water repellent and prone to rapid drying in spring.

As a result, seeding failures of sub-tropical grasses have been common, with establishment densities often less than 1 plant/m^2 or patchy establishment across the paddock. In general, the conditions for successful establishment of sub-tropical grasses have not been well understood by farmers, nor been well underpinned by science. However, the regular success by a small group of farmers and promising results in experimental trials indicated that successful establishment of sub-tropical perennial grasses was possible.

This sub-project was undertaken to gain a greater understanding of the seed biology and agronomic requirements for germination and establishment of sub-tropical perennial grasses, in order to develop a reliable establishment package for farmers and seeding contractors in south-western Australia. It combined laboratory and glasshouse studies in Sub-project 1 (to investigate optimum light and temperature conditions for germination and emergence, methods to overcome seed dormancy and the use of chemical priming and seed coating to enhance germination), with agronomic studies in the Field validation module to determine optimum sowing conditions (sowing times for different regions, sowing depth, machinery settings and weed control options).

3.2.4 Sub-Project 4 – Sown native species

Most of the work in this sub-project was conducted in the laboratory by Kings Park and UWA, in collaboration with Native Seeds Pty Ltd, in a RIRDC-funded project *"Grass roots – native perennial grasses for sustainable pasture systems* ". This project investigated use of a range of chemical and seed treatments to improve the establishment success of several native perennial grasses used by graziers, including *Austrodanthonia caespitosa, A. duttoniana, A. fulva, A. racemosa, Austrostipa nodosa, Chloris truncata, Elymus scaber, Enneapogon nigricans, Enteropogon acicularis, Microlaena stipoides* and *Themeda triandra.*

3.2.5 Sub-Project 5 – Naturally recruited native species

Progress on enhancing recruitment in native pastures has proven slow and difficult in the past. However, the substantial scale of the target environment in south-eastern Australia and the lack of economic alternatives for enhanced pasture improvement justified a persistent research focus. Most of this work in this sub-project was conducted in a MLA-funded PhD project at Charles Sturt University, Orange, titled "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*" (Thapa 2010). This project investigated a three-phase approach to develop simple low-cost management procedures to enhance the content of desirable perennial grass species. Management strategies were designed to encourage recruitment by: (i) encouraging seed set and shedding by desirable species; (ii) preparation of more suitable micro-sites for seedling recruitment; and (iii) identifying the best post-emergence tactics to aid seedling recruitment in the short to medium-term. Research was conducted on the native grasses, red grass (*Bothriochloa macra*), a C4 species, and wallaby grass (*Austrodanthonia* spp.), a C3 species to develop general principles applicable to a range of native grass species.

The sub-project was linked to some seed biology studies in sub-project 1 to provide an alternative avenue to firstly understand the seedling establishment requirements and then to investigate in the field how management can increase the number, duration or frequency of niche sites and/or recruitment events. The aim was to develop economical management practices to increase the opportunities for increased pasture productivity through recruitment in native pastures of desirable perennial grass species.

3.2.6 Sub-project 6 – Germination and establishment of tedera (additional activity)

This sub-project was added to the project, due to the increasing interest by the FFI CRC in tedera (*Bituminaria bituminosa* ssp. *albo-marginata*) as a new perennial pasture legume. This work was conducted as an Honours project through Curtin University, with co-supervision by Kings Park and DAFWA. The project conducted several laboratory studies to examine factors affecting germination, including optimum temperature and light conditions and ability to germinate under moisture and salinity stress. Seed dormancy issues and the potential use of plant signalling compounds to enhance germination were also examined in the laboratory. A glasshouse experiment was conducted to determine optimum sowing depth for emergence.

3.2.7 Field validation – integration between sub-projects

The Field Validation module was operated by the Department of Agriculture Western Australia (DAWA) and integrated the laboratory and glasshouse research on enabling seed technologies (undertaken in sub-project 1) with field and glasshouse agronomic research. The project provided funding for a Research Scientist (Dr Ron Yates) and a Technical Officer (split between two part time officers, John Titterington and Brad Wintle) to facilitate delivery of project outputs in target environments.

Micro-plots were used first to test the applicability and reliability of establishment methodologies that showed promise in the laboratory, in addition to agronomic research into aspects related to the establishment niche. Promising seed treatments and establishment technologies were then developed for wider scale field testing in farmer paddocks in target environments. This work was conducted in close collaboration with the Evergreen Farming group and the Saltland Pastures Association, as a ready-made conduit for technology transfer to informed producers.

3.3 Species evaluated

A total of 32 perennial species were evaluated in the project in laboratory, glasshouse or field studies (Table 1).

In Sub-project 2, nine chenopod species and two perennial grasses (puccinellia and tall wheat grass) were examined. However, initial studies showed there were no major barriers to germination and establishment of the two grasses, and so no further work was done on them. Most work was conducted on old man saltbush, as it showed the greatest propensity for direct seeding.

In Sub-project 3, six sub-tropical perennial grasses were examined. Some field trials also included the sub-tropical legumes lotononis (*Lotononis bainsii*) and Siratro (*Maroptilium atropurpureum*), but these were considered a lower priority and detailed studies on them were not conducted.

In Sub-project 4, 14 native perennial grasses were examined, as part of the RIRDC-funded project *"Grass roots – native perennial grasses for sustainable pasture systems* ", while in sub-project 5, recruitment studies were conducted on wallaby grass, *Austrodanthonia* species (these were not distinguished), and red grass (*Bothriocloa macra*).

Table 1. Perennial species studied in this project.

Species	
Scientific name	Common name
Saltland and rangeland species (Sub-project 2)	
Atriplex amnicola	River saltbush
Atriplex undulata	Wavy leaf saltbush
Atriplex nummularia	Old man saltbush
Atriplex semibaccata	Creeping saltbush
Atriplex bunburyana	Silver saltbush
Atriplex cinerea	Grey saltbush
Maireana brevifolia	Samll leaf bluebush
Maireana pyrimadata	Sago bush
Rhagodia preissii	Mallee saltbush
Puccinellia ciliata[1]	Puccinellia
Thinopyrum ponticum[1]	Tall wheat grass
Sub-tropical perennial grasses (Sub-project 3)	
Megathyrsus maxima	Panic grass
Pennisetum clandestinum	Kikuyu
Chloris gayana	Rhodes grass
Urochloa brachiaria	Signal grass
Setaria sphacelata	Setaria
Digitaria eriantha	Digit grass
Native grasses (Sub-projects 4 and 5)[2]	
Austrodanthonia caespitosa[3]	Wallaby grass
Austrodanthonia fulva[3]	Wallaby grass
Austrodanthonia pilosa[3]	Wallaby grass
Austrodanthonia racemosa[3]	Wallaby grass
Austrodanthonia richardsonii[3]	Wallaby grass
Austrodanthonia setacea[3]	Wallaby grass
Bothriochloa macra[3]	Red grass
Chloris truncata	Windmill grass
Chloris ventricosa	
Dichanthium sericeum	
Enteropogon acicularis	
Heteropogon contortus	
Microlaena stipoides	Weeping rice grass
Themeda triandra	Kangaroo grass
Tedera (Sub-project 6)	
Bituminaria bituminosa	Tedera

[1]No germination issues were found with puccinellia or tall wheat grass, so no further studies were undertaken with them

[2]Germination and establishment studies of native grass species were mainly conducted through the RIRDC-funded project *"Grass roots – native perennial grasses for sustainable pasture systems* "

[3]Recruitment processes of *Austrodanthonia* species and *Bothriocloa macra* were studied in the MLA-funded PhD project "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*" (Thapa 2010).

3.4 Experimental methodology

Details of the methodologies used in individual experiments are presented in Attachment 1 (establishment of sub-tropical perennial grasses), Attachment 2 (establishment of chenopod shrubs), Attachment 3 (establishment of sown native species), Attachment 4 (recruitment of native grasses) and Attachment 5 (establishment of tedera).

4 Results and Discussion

4.1 An establishment package for sub-tropical grasses

The project was very successful in developing a reliable establishment package for sub-tropical grasses in south-western Australia. The package is applicable to Rhodes grass (*Chloris gayana*), panic grass (*Megathyrsus maximum*), signal grass (*Urochloa brachiaria*), setaria (*Setaria spp.*), digit grass (*Digitaria eriantha*), Bambatsi panic (*Panicum coloratum*) and kikuyu (*Pennisetum clandestinum*), either sown alone or in mixtures. It is also likely to be relevant to other areas with similar climates and soils.

Adoption of the establishment package has been rapid, due to close interaction with the Evergreen group, catchment councils and seeding contractors. At the commencement of the project, commercial seeding failures were high and establishment was often patchy, with a mean establishment density in the order of 1 plant/m^2. The greater understanding of the key factors for establishment success among farmers and seeding contractors has given rise to an expectation of achieving establishment densities of > 10 plants/m^2. The estimated area sown to sub-tropical grasses in WA has increased from 120,000 ha to 150,000 ha since the commencement of the project (P. Sanford, unpublished data). Greater confidence in achieving establishment success has been a key driver in this increased adoption.

The establishment package is based on ten steps. It aims to achieve a perennial grass density of 8-10 well established plants/m^2 surviving though to the first autumn after sowing, which was shown in the project to be a desirable benchmark.

Full details of the experimental results and conclusions used to formulate the package are provided in the Technical Bulletin "*Establishment of sub-tropical grasses in south-western Australia*" (Attachment 1). A Farmnote "*Establishing sub-tropical perennial grasses*", which gives the essential steps to establishment success, has been published and distributed to 600 subscribers of the Evergreen newsletter (Attachment 8).An Extension bulletin, "*Establishment guide for sub-tropical grasses – key steps to success*", which contains more background information and some farmer case studies, has also been prepared for publication (Attachment 7).

The key steps for reliable establishment of sub-tropical grasses are outlined below.

1. Plan ahead

Plan a year ahead and reduce weed seed-set by grazing and spray-topping, especially for difficult-to-control weeds. For exposed paddocks prone to wind erosion, consider sowing a cereal to provide stubble for soil stability. Commence control of rabbits and kangaroos if they are a potential problem.

2. Purchase good quality seed

Select species and varieties suited to your district, soil type and intended use. Refer to Moore *et al.* (2011*a*) for best sowing options for the northern agricultural region and to Moore *et al.* (2011*b*) for the south coast.

Germination of commercial sub-tropical grass seed batches typically varies from 10–60%. Kikuyu (commonly >80%) is an exception. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. Several seed companies coat their seeds. While this helps with handling and flow of fluffy seeds in machinery, it reduces the number of viable seeds per kilogram.

Seed dormancy

Panic grass, setaria and signal grass have a high proportion of seeds, referred to as *fresh seeds*, which remain dormant for 6–10 months after harvest. Consider purchasing these species the year before seeding and storing under dry conditions. Rhodes grass and kikuyu do not have such dormancy.

3. Control weeds and insects prior to sowing

A weed-free seed bed is essential as sub-tropical grass seedlings are weak competitors. Weed control strategies should aim to minimise wind erosion, particularly on exposed sites with sandy soils. One strategy is to use a selective broadleaf herbicide 6 weeks from sowing, followed by a general knockdown herbicide 2 weeks from sowing. This allows grass residues to bind the soil and reduce erosion risk.

In paddocks with a low grass density, a single knockdown herbicide 2 weeks before sowing can be used, but higher rates than autumn-winter applications will be needed to kill difficult-to-control weeds.

Two knockdown sprays (6 weeks and 2 weeks before seeding) result in good weed control but leave the paddock prone to wind erosion (unless stubbles have been retained). It also reduces the amount of winter grazing and the ability to change plans if sowing conditions deteriorate.

Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

4. Sow into moisture in late winter – early spring

The best sowing times for the main regions suited to sub-tropical grasses in WA are:

- Dongara—Kalbarri districts—early to late August
- Perth—Eneabba districts—mid-August to early September
- South coast—early September to early October

This coincides with sufficient soil temperatures for germination and the likelihood of sufficient moisture for good root development before summer.

If soil moisture conditions are unfavourable for sowing, defer pasture establishment until the following year.

5. Set-up the seeder for the best result

Successful establishment of sub-tropical grasses can be achieved with a range of seeding machinery and configurations (tynes, discs) provided the machine:

- forms stable furrows that scalp away non-wetting sand, remove weed seeds and harvests rainfall
- uses press wheels to provide good seed contact with moist soil

- has a wide row spacing (typically 50-60 cm)

Formation of furrows

Sowing into furrows is important to scalp away non-wetting sand, remove weed seeds from near the perennial grass seedlings and harvest rainfall. Furrows capture rainfall and increase seedling survival, particularly in dry springs. They act to turn relatively small rainfall events into useful soil moisture for growth and survival. For example, 3-5 mm of rain can effectively become 10-15 mm for seedlings in the bottom of a furrow.

Furrow depth can be varied according to seasonal conditions. The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting.

Furrow shape should be designed to minimise furrow collapse and sand in-fill, as this causes burial of the seed, which can markedly reduce seedling emergence. Wide furrows with sides that are not too steep are less prone to collapse and sand in-fill.

Seeding without furrows is risky, as it relies on favorable spring-summer rainfall, and is more likely to result in poor or patchy establishment, particularly in years with low spring and summer rainfall.

Press wheels

Press wheels provide good seed contact with soil moisture and lead to more reliable germination. They should press soil around the seed in the furrow bottoms and minimise sand in-fill from the furrow sides. An easy sowing method is to drop seeds into the bottom of furrows and use press wheels to push them below the soil surface. This system positions seeds well for germination, but strong press wheel action is essential to give good seed-soil contact.

The contour of the press wheels should fit within the furrow shape to minimise sand in-fill. The base of the furrow should be wider than the press wheels. Rounded or flat-bottomed wheels tend to produce a better result than angular, narrow wheels, while ”W”-shaped press wheels are the least effective. It is also important to ensure that press wheels are correctly aligned with the furrows and that the arms allow consistent tracking along furrows.

Optimum row spacing

Optimum row spacing appears to be 50-60 cm. This ensures a sufficiently wide inter-row so that any soil movement during sowing from one furrow does not cause sand in-fill into adjacent furrows. It also gives sufficient space for annual pastures to grow between the rows in the cooler months, resulting in a good balance between perennial and annual pasture components.

A wider row spacing can be used in low rainfall areas to reduce competition for soil moisture. Row spacing can also be adjusted to change the balance between winter-spring feed from annual pastures in the inter-row versus potential out-of season production from the perennial grasses.

Close row spacing, such as that achieved with a conventional seeder, can result in greater competition for water, resulting in small, stunted plants and reduced growth following out-of-season rainfall.

Sow different grass species in alternate rows to reduce competition

In mixtures of sub-tropical grasses pasture composition can be dominated by the species with higher seedling vigour. Rhodes grass has the highest seedling vigour of the commonly grown sub-tropical grasses and often out-competes panic grasses and other species when sown together. To overcome this, Rhodes and panic grasses can be sown in alternate rows. Simple dividers can be used in the seed box to allow species to be sown in alternate rows.

6. Sow 2–5 kg/ha of seed

Calibrate the seeder to ensure the correct amount of seed will be sown.

Sow 2-5 kg/ha of seed, depending on seed quality and whether the seed is coated. As a guide, for uncoated seed with 40% germination, 2 kg/ha is sufficient to give a good seedling density. Higher rates should be used for seed of lower germination. Higher rates are also required for coated seeds to sow the same number of seeds per area.

Kikuyu seed normally has high seed quality (> 80% germination) and sowing at 1 kg/ha is usually adequate. However, full groundcover will be achieved more rapidly by sowing at 2 kg/ha.

With light, fluffy seeds like Rhodes grass, it is often necessary to use a carrier if the seed is not coated. Use a low rate of fertiliser (e.g. 20-25 kg/ha) mixed with the seed for better flow through the machinery.

7. Sow at a depth of 5–10 mm

Sub-tropical grasses require shallow should be sown to a depth of 5–10 mm. **Seeds sown too deep will not emerge**. A common method is to drop seeds in the bottom of furrows and press them in with press wheels. When correctly adjusted, a small proportion of sown seeds are usually visible on the soil surface. The bright colour of coated seeds makes checking seeding depth relatively easy. Seeding depth should be checked regularly to ensure that the majority of seeds are being placed 5-10 mm below the surface in the bottom of furrows. This should be done at normal operating speed.

Sowing directly onto the soil surface is unreliable.

8. Don't sow too fast

Tractor speed can impact on establishment success. Driving too fast can cause excessive soil movement, reducing the accuracy of seed placement. Sand in-fill increases and furrows can collapse, resulting in a deeper effective seeding depth and poor resulting establishment. Optimum speed varies with the type of machinery, but from experience speeds of 5-10 km/h generally give a good result.

Some machinery modifications can be made to enable sowing speed to be increased. For example, some producers have added rubber guards to prevent soil from one furrow being thrown into adjacent furrows.

To ensure the majority of seeds are being placed 5-10 mm below the surface sow at normal operating speed and check the results. Check seed placement regularly and make adjustments if needed.

9. Conduct post-seeding checks

There are a number of post-seeding checks that should be conducted to ensure successful perennial grass establishment.

Determine establishment success

Establishment success and pasture composition can be gauged from the time emerging grasses have three leaves. Good establishment should result in continuous lines of perennial grasses in each seeding row across the paddock. Plant density can be determined by counting the number of plants per metre of seeding row. Initial density should be at least 10 plant/m^2 for sowing to be considered successful. Pasture composition can be determined by estimating the relative number of each species.The sown perennial grass seedlings can be identified by comparing with the identification guides in Wintle *et al.* (2010)

Control insect pests

Apply a residual insecticide at sowing or with the last knockdown herbicide to control insects. Monitor the paddock regularly for insect damage, especially over the first 8 - 10 weeks when grass seedlings are emerging and are most vulnerable to insect attack. Control cutworm and webworm caterpillars, redlegged earth mite, Rutherglen bugs and aphids if present.

Control summer growing weeds

Summer growing weeds compete strongly with sub-tropical grass seedlings for soil moisture. Control of these weeds will optimise establishment of sown sub-tropical grasses. Broadleaf weeds such as Afghan and paddy melons, wireweed and fleabane can be readily controlled with a number of broadleaf herbicides, provided they are actively growing.

Couch grass is a highly competitive summer-active perennial grass that will out-compete the more desirable sown perennial grass seedlings during the establishment phase. Care needs to be taken with couch grass, as it often recovers from a knockdown herbicide applied in winter. However, once established, the sown sub-tropical grasses are more productive and compete strongly with couch grass.

Special consideration needs to be given to erosion-prone soils when perennial grass seedling density is low (< 5 plants/m^2). Summer weeds should be retained in such situations to reduce the erosion risk.

Control kangaroos and rabbits

Kangaroos and rabbits can damage or kill young sub-tropical grass plants, so good control is essential. Unrestricted grazing can up-root establishing plants and increase stress over summer, which may reduce their persistence. In particular kangaroos will travel considerable distances to graze sub-tropical grasses, when little other green feed options are available.

10. Defer grazing until grasses are well established

Careful grazing management over the first summer is critical to ensure a strong perennial stand in subsequent years. Sub-tropical grass seedlings have a weak primary root system and as a result are susceptible to up-rooting and grazing damage over the first summer. Uncontrolled grazing during this time will also deplete carbohydrate reserves which can result in plant death.

The timing of the first grazing will vary depending on seasonal conditions and how well the plants have grown. If late spring or summer rainfall has produced vigorous growth, the perennial grasses can be lightly grazed from early January. However, if very little or no summer rainfall has fallen, the first grazing will need to be deferred until after the break of season.

The first grazing should be deferred until the perennial grasses are well established and well anchored.

Before the first grazing assess whether plants are firmly anchored by testing how easily they can be pulled out by hand. What appear to be relatively large plants can have a surprisingly small root system and in that case premature grazing would have a significant negative impact on the stand density with plants being uprooted and killed. Rhodes grass has long runners (stolons) that do not root down in dry soils and is, therefore, particularly susceptible to grazing damage in the first summer (test by pulling on the stolons).

If the plants are well anchored then a light grazing will encourage tillering. Monitor the perennial grasses while stock are in the paddock to ensure they are not being up-rooted or over-grazed.

On-going grazing management will vary with the perennial grass, grazing animal and time of year. Most perennial grass paddocks can be largely set-stocked by cattle during the growing season, but they will require some form of rotational grazing outside of the annual growing season. Kikuyu is highly grazing tolerant and can be set stocked for long periods by sheep or cattle, but leading producers have found large increases in productivity by rotationally grazing kikuyu pastures.

4.2 Identification of sub-tropical perennial grass seedlings

It was seen that there was a need for farmers and seeding contractors to be able to recognise emerging seedlings to determine the success or otherwise of their seeding operation and to distinguish sown plants from grass weeds. Consequently, a photographic study was made on germinating grasses seedlings in the glasshouse. This culminated in publication of the Technical Bulletin "*Identifying sub-tropical grass seedlings*" (Wintle *et al.* 2009), which shows photographs and provides descriptions of seedlings of Rhodes grass, panic grass, signal grass, digit grass, setaria, kikuyu and Bambatsi panic grass. This has been published and distributed to 600 subscribers of the Evergreen newsletter (Attachment 6).

4.3 Direct seeding of old man saltbush

The project was very successful in developing a direct seeding package for old man saltbush (*Atriplex nummularia*), the most widely planted saltbush species across southern Australia. It is suited to revegetating slightly- to moderately-saline land (EC_e values at 0-30 cm depth between 2 and 8 dS/m) and to rangeland environments. The package is designed for seeding with conventional farm seeding equipment, with some modifications for wide row spacings and depth control.

A key finding was subspecies and genotypic differences for ability to establish from direct seeding within *Atriplex nummularia*. Subspecies *nummularia* was found to be suited to direct seeding, but genotypic differences were found within this subspecies for expression of this trait; seed of types that readily established from seed (Emergent types) have been included in the FFI CRC saltbush breeding program. On the other hand, subsp. *spathulata* was found to be unsuited to direct sowing; this subspecies has also been found to be unpalatable to grazing sheep in other FFI CRC studies.

This technology now provides landholders with a reliable, low-cost method of establishing old man saltbush. Costs are expected to be around ($100-150/ha), compared to the previously more reliable, but more expensive method of transplanting nursery-raised seedlings of more than $450/ha.

Full details of the experimental results and conclusions used to formulate the package are provided in the Technical Bulletin "*Direct seeding of chenopod shrubs for saltland and rangeland environments*" (Attachment 2). A Farmnote "*Direct seeding of old man saltbush*" (Nichols *et al.* 2010), which gives the essential steps to establishment success, has also been prepared for publication (Attachment 3).

The direct seeding package for old man saltbush is based on the nine steps outlined below.

1. Select appropriate paddocks

Good site selection is critical to successful establishment. The most appropriate paddocks are those with slightly- to moderately-saline land (EC_e values at 0-30 cm depth between 2 and 8 dS/m).

Productivity is markedly reduced on extremely saline land (EC_e > 32 dS/m). *Atriplex nummularia* is susceptible to waterlogging, so avoid areas prone to prolonged waterlogging. Sites with 50-100% annual ryegrass and some slender ice plant are generally ideal. If the site has samphire or is bare and scalded, then it is too saline. If the site has substantial sea barleygrass, then it is too waterlogged. Duplex soils with sandy loam over clay are the easiest to establish, as the sandy surface allows salts to leach through the soil following winter rains, creating a favourable environment for germination. More details on appropriate site selection are provided in Barrett-Lennard *et al.* (2003).

2. Prepare sites for optimum establishment

A weed-free seed bed is essential, as *A. nummularia* seedlings are weak competitors. Plan a year ahead if possible and reduce weed seed-set in the year before sowing by grazing and spray-topping, especially for ice plant and annual ryegrass. Commence control of rabbits and kangaroos if they are a potential problem.

A good weed control strategy is to use two knockdown sprays (4-6 weeks and 1-2 weeks before seeding). Cultivation prior to sowing can make the surface too uneven for precise sowing. Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

3. Sow the best seed

Germination of *A. nummularia* seed batches is generally low, and typically varies from 5–20%. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. The following steps will help maximise use of high seed quality.

Ensure seed is subspecies nummularia

This project indicates that subspecies *nummularia* is best suited to direct seeding. The alternative subspecies, subsp. *spathulata*, appears to have different requirements for germination and does not readily establish from direct seeding (see Section 6). Other studies in the “Enrich” project of the FFI CRC have also shown that subsp. *nummularia* is much more palatable to grazing sheep than subsp. *spathulata*.

Ensure seed is fresh

Use seed harvested within the previous 6 months that has been stored in a cool, dry environment.

Use large heavy fruits

Large and heavy fruits should be selected for sowing, as they are more likely to contain mature, viable seeds. Small fruits, on the other hand, are more likely to be empty or contain undeveloped embryos. If there is a large range in fruit size, grade off the smaller fruits.

Retain bracts

Do not remove the bracts surrounding the fruit, as establishment success in the field has been higher for seed with bracts, compared to seed with their bracts removed (see Section 4.1).

4. Sow into moisture in late winter-spring

The ideal time for sowing is a compromise between soil temperatures being warm enough for germination and the likelihood of there still being sufficient moisture for good root development before summer. On saline land, sowing into moisture in late winter-spring also means salts are more likely to have been flushed from the soil surface. This time of year also allows good control of winter weeds and insects prior to sowing.

The ideal sowing window is earlier in more northerly districts than more southerly ones. For example, the best sowing times for different regions in Western Australia are:

Northern agricultural region - early to late August

Central and eastern wheatbelt - mid-August to early September

Southern agricultural region - late August to late September

In areas with more reliable spring and summer rainfall, such as northern New South Wales, the sowing window will be more extended.

Seeds should be sown into a moist seedbed, or if there is a strong likelihood of rain.

Although *A. nummularia* is salt tolerant, its germinating seeds are susceptible to salinity and will not germinate if the soil surface is too saline. Rainfall leaches salts from the surface, providing a relatively fresh environment for germination. If the soil surface in a saline environment is dry, it is quite likely to be too saline for germination of *A. nummularia*.

If the area to be sown is waterlogged, sowing should be deferred until later in spring, or to the following year.

5. Aim to establish at least one plant per 2 m of row

Use a sowing rate of ~10 fruits/m (if germination rate is 15%). This should provide at least one plant every 2 m, allowing for losses of ~60%. Higher rates should be used for seed of lower germination.

6. Set-up the seeder for the best result

A standard Massey Ferguson combine seeder (or something similar) can be used to direct seed old man saltbush, with some minor modifications. The simplest way is to remove tynes not needed for sowing. Small boxes (one for each seeding tyne) can be attached to the seeding box to hold the small quantity of seed required, with hoses attached to seeding tynes. Seeders should also have the following features.

Formation of furrows

Tynes should create furrows to capture rainfall and increase seedling survival, particularly in dry springs. Furrow formation also scalps away non-wetting sand and removes weed seeds. Furrows should be formed to minimise soil in-fill and should be broad to increase the area of rainfall capture.

The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting, provided furrow collapse and soil in-fill is avoided, as this causes burial of the seed, which can markedly reduce seedling emergence

Press wheels

Press wheels provide good seed contact with soil moisture and reduce in-fill of soil into the furrows. They should press soil in the furrow bottoms and minimise soil in-fill from the sides. Flat-bottomed wheels give the best results.

Row width

Calculate the desired width between saltbush rows for each seeding pass and adjust the width of seeding tynes accordingly, removing non-seeding tynes. Typical row widths for alleys of double or triple rows are 1 m. A stand of saltbushes 1 m x 1 m apart can be regarded as being very dense.

7. Sow to a depth of 5–10 mm

Atriplex nummularia requires shallow seeding to a depth of 5–10 mm. Seeds sown too deep will not emerge. The simplest method is to drop fruits in the bottom of furrows and press them in with press wheels. When correctly adjusted, this will leave a small proportion of fruits visible on the soil surface. Sowing directly onto the surface is unreliable.

8. Control weeds and pests (insects, kangaroos and rabbits)

Summer growing weeds compete strongly with saltbush seedlings for soil moisture. Control weeds with appropriate herbicides to maximise establishment. Monitor the paddock for insect damage, especially over the first eight weeks and control if needed. Good control of kangaroos and rabbits is essential to protect young seedlings.

9. Defer grazing until seedlings are well established

The first grazing of *A. nummularia* bushes should be deferred until they are well established and actively growing. This will vary with seasonal conditions and may not be until after the break of the next season. Ensure plants are firmly anchored before introducing animals.

4.4 Direct seeding of *Rhagodia preissii* (mallee saltbush)

Limited field trials suggest the techniques used for old man saltbush can also be used for direct seeding *Rhagodia preissii*. However, this is based on the success of just one trial in the northern agricultural area of Western Australia. This is the region where *R. preissii* is being promoted, but further trials are required to demonstrate repeatability of establishment in more southerly areas, with cooler soil temperatures, and on other soil types.

4.5 Direct seeding of other chenopod species

4.5.1 Other *Atriplex* species

This project was not able to develop reliable establishment packages for other *Atriplex* species including *A. amnicola* and *A. undulata*. The project had limited success with these species, which presently work well with the “Mallen” niche seeder. Further work is needed to understand the triggers for seedling emergence before these species can be direct-seeded with conventional machinery.

4.5.2 *Maireana brevifolia* (small leaf bluebush) and *M. pyramidata* (Sago bush)

Direct sowing of *M. brevifolia* and *M. pyramidata* appears to be problematic in much of southern Australia. The main difficulty is their requirement for warm temperatures (~30°C) to germinate. These temperatures do not normally occur until November in southern Australia, by which time the winter growing season has generally ceased. This precludes the direct seeding of these species without the aid of irrigation. An exception to this would be areas with more reliable summer rainfall, such as northern New South Wales. Here, sowing could be deferred until late spring-early summer.

Field observations indicate widespread recruitment of new *M. brevifolia* seedlings from surrounding bushes, most likely after episodic summer rainfall events (P.G.H. Nichols and E.G. Barrett-Lennard, unpublished data). This suggests an alternative and potentially cheap method of establishing *M. brevifolia*, if it is already present in the area, is to encourage natural recruitment of seedlings from seed produced on surrounding bushes. However we need ways to get at least small numbers of viable plants into virgin sites. One way of doing this could be to transplant a low density of nursery-raised seedlings which could then act as a seed source for natural recruitment. It is likely *M. pyramidata* could be established in a similar way. In order to devise strategies to increase recruitment of new seedlings, there is clearly a need for studies to better understand the ecology of *M. brevifolia* and *M. pyramidata* and their cues for germination. It may also be possible to identify populations or genotypes of both species with lower temperature requirements for germination, that make them more suited to direct sowing in late winter or early spring.

4.6 Native perennial grass establishment

The use of dormancy breaking treatments and germination stimulants, such as smoke water, gibberellic acid, heat treatment and manipulation of florets, improved germination in the laboratory of several native grass species from 5% to >90% in some cases. One or more of these treatments improved germination of *Austrodanthonia* species, *Chloris truncata*, *Themeda triandra*, *Dichanthium sericeum*, *Enteropogon acicularis* and *Microlaena stipoides*. Species differences were also found for after-ripening and storage treatments. The translation of these treatments to establishment in the field requires further research.

Seed quality is a significant issue with native grasses, particularly if collected from wild stands. This project highlighted two techniques (X-ray analysis and air separation) that provide rapid means to improve seed quality for end users.

An understanding of the species/accession specific germination biology has allowed significant improvements in germination performance of native grass seeds. The use of dormancy breaking treatments and germination stimulants has further improved germination performance of several species of native grass seed in laboratory environments, for example:

- *Austrodanthonia* species respond well to smoke water treatment at 1% solution, which increases germination from 26% to 96% if seeds are cleaned
- *Chloris truncata* germination can be increased in some ecotypes from 5% to 48% when treated with gibberellic acid and further increased to 86% if also heated at 100°C for 30 minutes
- this heat treatment will also increase germination of *Themeda triandra* seeds from 5% to over 30%
- Gibberellic acid enhances germination of *Dichanthium sericeum* from 9% to 74% if the florets remain intact; if the caryopses are removed >90% germination can be achieved without further treatment
- *Enteropogon acicularis* germination was improved from 48% to 99% by removing the caryopses from the enclosing floret structures without the need for germination enhancing chemicals
- *Microlaena stipoides* seeds germinate to between 90 and 100 % when fresh either as intact florets or as cleaned seeds without germination stimulants but gibberellic acid may increase the rate of germination.

The use of stress signalling compounds appears to have a broader effect on native grass species, with salicylic acid and kinetin improving germination under water and salinity stress in many species. Response to drought stress was species specific however *M. stipoides* appears to be the most resilient native grass tested, at least at the germination phase.

After-ripening and storage treatments highlighted species differences. However when stored under ideal conditions (cool and dry), there appears to be no negative effect of storage in *A. caespitosa* and *M. stipoides* after 6 and 12 months respectively.

Further experimental details are given in the attached technical bulletin (Attachment 3).

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species/accessions tested. The approach used in this report can be readily adapted to industry to improve the quality of the end product. This quality increase will hopefully facilitate broad scale adoption of native grasses in Australia.

4.7 Recruitment of desirable perennial grasses in native pastures

This project was successful in developing a low-cost pasture management package to increase recruitment of desirable perennial grass species in native pastures. The research used to develop these guidelines was conducted in central New South Wales on the native grasses, wallaby grass (*Austrodanthonia* spp.) and red grass (*Bothriochloa macra*), as part of a MLA-funded PhD project "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*" through Charles Sturt University (Thapa 2010).

The key findings from this research were that successful seedling recruitment occurs in early autumn, provided the following conditions are met: (i) adequate quantities of germinable seed are available (following removal of stock from spring to promote flowering and seed set); (ii) a suitable rainfall event after seed maturation and shedding results in a 7-15 day period of moisture in the top 50 cm of soil; and (iii) an appropriate micro-site is present for seedling emergence (light scarification can help on soils that are not self-mulching). Insecticides can be used to control seed-harvesting ants and sub-lethal herbicide doses can be used at seed maturity to weaken plant competition prior to recruitment. Climate analyses indicated that soil moisture conditions in February-March should be favourable for seedling recruitment in most years.

These results have been used to develop a management package for farmers to enhance the recruitment of desirable perennial grass seedlings within existing pastures. The techniques can be used to significantly increase the perennial grass content of pastures across south-eastern Australia at low-cost, with substantial impacts on medium to long-term profits and environmental benefits.

Full details of the experimental results and conclusions used to formulate the package are provided in Roshan Thapa's PhD thesis (Thapa, 2010). A Technical bulletin "*Low cost rehabilitation of perennial grass pastures by managing seedling recruitment*", which outlines the pasture management package to increase desirable perennial grass recruitment and the key supporting research results, has been prepared for publication (Attachment 4). An advisory note, which gives the essential steps to manage pastures for recruitment of native grasses, has also been prepared for publication (Attachment 10).

Although the research work was conducted in central New South Wales, the management package is applicable to farmers and their advisors in the 600 - 800 mm rainfall zone throughout south-eastern Australia. The research was mainly aimed at recruitment of seedlings within native pastures, but the principles can also be applied to seedling recruitment in sown perennial grass pastures.

The key steps for low-cost pasture management to increase recruitment of desirable perennial grass species in native pastures are outlined below.

4.7.1 Principles

Allowing new seedlings of desirable perennial grasses to establish within existing swards is a viable practice that farmers can use to restore pastures to a more productive state (~60% desirable perennial grasses), without the need for re-sowing. The investment costs are considerably less and restored paddocks are more profitable over time.

Work with one or two paddocks at a time, rather than the whole farm, in the first instance. Start by selecting a paddock with a moderate content of desirable perennial grasses (around 20-30% is ideal), rather than the poorest paddock. Better paddocks provide faster rates of improvement and quick seed production. The seeds are then available for harvesting and redistribution to poor paddocks.

The paddock has to be free of major weed problems. Control annual grasses and seasonal weeds, with herbicides and grazing, in the year before pasture improvement is attempted. Desirable perennial grasses can tolerate low rates of herbicide applications. Having a paddock relatively free of weeds is a prerequisite for success of this management strategy.

Few seeds of perennial grasses generally remain in the soil from previous seasons. Therefore, emergence of new seedlings depends predominately on seeds set in the current year. Grazing needs to be managed to encourage flowering and seed set.

Rest paddocks (i.e. remove stock) to maximise flowering and seed set creates sward conditions that enhances emergence of seedlings. Standing plant material (even if ~80% dead) is more useful than litter (dead plant material lying on the soil surface) in most instances. More than 2000 DM kg/ha of litter is detrimental to emerging seedlings.

Determine basic soil characteristics of the paddock, including nutrient status, pH and texture. Fertilisers should be applied to address nutrient deficiencies that affect seedling growth, especially in low fertility areas. Phosphorus, in particular, is important for early root growth of seedlings. Roughing up the soil surface by harrowing, especially on soils that are hard setting, increases the locations where seeds can lodge and germinate, thus enhancing emergence of new seedlings.

Consider use of an insecticide to control seed harvesting ants if they are a problem. This is often the case with phalaris in sown pastures.

Rainfall events in late summer or early autumn that result in at least 10 days of moist soil in the top 50 mm triggers successful seedling emergence -15 days of surface soil moisture is ideal. Seedling emergence is not hindered by 2 days where the soil surface is dry during this period.

Some establishment of new seedlings will occur in most years, but resting paddocks from late spring through to late March and following the management strategies in this bulletin will maximise the opportunities for seedling emergence.

In the first year of applying these management tactics, a conservative rate of improvement in perennial grass content may be 0-20% of the total herbage mass produced per year. It could take 4-5 years to reach a target of 60% desirable perennial grasses, if there is an average of 10% improvement per year.

4.7.2 Seasonal management tactics

Spring

Do not let the selected paddock get overgrown in early spring. Aim to have the herbage mass around 1500-2000 kg/ha of dry matter when the paddock is locked up in late spring to allow plants to flower and set seed.

Early – mid summer

Allow seeds to mature and fall onto the soil surface. Monitor seasonal conditions in December and January. Continue the summer rest if rainfall for these months is >40% of average rainfall.

If the season is poor (<40% average rainfall in these months), defer setting up the paddock for seedling establishment until the next year. Even if rains in late February provide the right conditions for recruitment, seed set will have been minimal and feed will be needed for livestock.

Consider applying an insecticide if seed harvesting ants are present in high numbers. Phalaris is of the most concern.

On hard-setting soils carry out mild soil disturbance to provide micro-environments for seeds to lodge and germinate. This can be done using harrows dragged behind a vehicle.

Late summer - early autumn

Look for new perennial grass seedlings within 2-3 weeks of a significant rainfall event in late February or March.

Autumn - winter

Once seedlings cannot be pulled out easily by hand, the paddock can be lightly grazed until ~1500 kg/ha of dry matter remains.

Guidelines developed for managing sown pastures are also applicable to managing pastures enhanced through natural establishment.

4.7.3 Benefits

Costs involved in improving paddocks through natural seedling recruitment will be substantially less than re-sowing a new pasture, as few inputs are required. In a good season resting one or two paddocks will have little impact on the feed supply needed to carry livestock on the farm. Soil disturbance using harrows may cost ~$20/ha and is only necessary for hard setting soils. Insecticides and herbicides will incur some additional costs if they are needed. However, this will be considerably less than the ~$300/ha needed to sow a new pasture.

Once a stable target of 60% content of desirable perennial grasses is reached, increased livestock production will result, either through greater carrying capacity or more production per head. Estimates for some native grass pastures in New South Wales suggest livestock production can be more than doubled in the medium term, depending on soil characteristics.

It is useful for farmers to rest some paddocks to enable seedling establishment if suitable seasonal conditions prevail. If the conditions turn dry over summer then emergence of new seedlings is less likely. Rested paddocks can then be grazed, as feed is likely to be limited at this time. In wet summers there is usually no shortage of feed and resting paddocks for longer periods is not likely to cause major difficulties.

This information now provides farmers with a low-cost approach to pasture improvement for those large parts of the landscape where there are still some desirable perennial grasses.

4.8 Germination of tedera (*Bituminosa bituminaria* ssp. *albo-marginata*)

This project was successful in developing a preliminary establishment package for tedera for testing in the field. The research used to develop these guidelines was conducted as part of an Honours project "*Germination ecology of* Bituminaria bituminosa *var.* albo-marginata *and its suitability to the Mediterranean-type climate of Western Australia*" through Curtin University and co-supervision by Kings Park and DAFWA (Beard 2009).

The key finding from this research is that there do not appear to be any major barriers to successful germination and establishment of tedera in the field, provided soil moisture conditions are adequate. An establishment package for tedera has been developed, on the basis of experimental results from this project and from common agronomic practices used for other crops and pastures.

Tedera accessions were found to be relatively similar in regard to seed characteristics. Its seeds possess a non-deep physiological dormancy characteristic that restricts protrusion of the radical through the seed testa. Manipulation of the seed coat, by surgical cutting, scarification or threshing, was relieved this restriction and increased germination percentages by up to 72%. Tedera was found to possess after-ripening seed dormancy of three months from maturation, but this is not likely to cause problems for sowing, as seed harvested in early summer will not have dormancy issues if planted in early winter.

Tedera germinates readily in temperatures ranging from 5°C to 35°C, with optimum germination at 15-25°C. This implies tedera is well suited to germination in the autumn-winter period of southern Australia. Tedera had no germination requirement for light at 15°C and 20°C. Tedera germinated more readily under moisture stress than lucerne, but its germination did not differ from lucerne at any salinity level.

Tedera has a relatively large seed. A sowing depth trial showed that tedera seedlings readily emerge when sown from a depth of 2- 8 cm. The majority of seeds also emerged from 10 cm, the deepest seed placement tested, although there was a delay in cotyledon emergence. However, sowing seeds on the surface caused high mortality, with a low seedling emergence resulting. This result implies that conventional seeding equipment can be used to sow tedera and that it will emerge at high rates from a seeding depth ranging from 2-8 cm.

Priming of tedera seeds with the plant signalling compounds, smoke water, KAR_1, and GA_3 and priming with water did not produce higher germination percentages than un-primed controls, bit smoke water and KAR_1 did increase germination rates. KNO_3, on the other hand, inhibited germination, while ethylene produced different results between accessions. These effects need to be tested in the field, but the ready germination and lack of major dormancy issues in tedera, suggest chemical priming is unlikely to be beneficial.

A Technical bulletin, "*Establishment of tedera* (Bituminaria bituminosa *var.* albo-marginata)", outlining the establishment package for tedera, has been prepared for publication and is attached as Attachment 5). Full details of the experimental results and conclusions used to formulate the package are provided in this bulletin.

The key steps for establishment of tedera are outlined below.

1. Select the paddock for sowing

Plan a year ahead of sowing, if possible, and reduce seed set of annual weeds in spring by grazing and spray-topping

2. Use good quality seed

Use recently harvested seed where possible. Seed harvested in early-mid summer should not have significant dormancy issues for sowing in late autumn or winter. Seed longevity studies have not been conducted, but seed older than two years should be germination tested to ensure viability.

3. Prepare a weed free seed bed prior to sowing

Control summer weeds and germinating weed seedlings at the break of season with knockdown herbicides or cultivation to prepare a weed-free seed bed. Paddock preparation for tedera should use similar good agronomic practice as for sowing crops or other pasture species.

4. Sow into a moist seedbed in autumn or early winter

Tedera is best suited to sowing in late autumn or early spring – a similar time to annual pastures and crops. It should be sown into moist soil, The option of dry sowing has not been tested, but it is likely to be more risky.

5. Inoculate with the appropriate rhizobium strain

If tedera is commercialized, it is likely to need its own special rhizobium inoculant to fix nitrogen. Either lime pellet the seed or add granular inoculants to the seed box (if available). Rhizobia for tedera are under development.

6. Drill seed in with conventional seeding machinery

Conventional seeding machinery can be used to sow tedera. Its relatively, large, robust seed means that particular care is not needed to ensure sowing depth is precise. Sowing depth should be within the range of 2-8 cm. Some soil cover is required, so do not drop seeds onto the soil surface.

Sowing into furrows is important to scalp away non-wetting sand, remove weed seeds from near the tedera seedlings and harvest rainfall. Furrows capture rainfall and increase seedling survival, particularly in dry periods soon after sowing. They act to turn relatively small rainfall events into useful soil moisture for growth and survival. For example, 3-5 mm of rain can effectively become 10-15 mm for seedlings in the bottom of a furrow.

Press wheels could be used to provide good seed contact with soil moisture in the furrow bottoms.

7. Control weeds and pests (insects, kangaroos and rabbits) post-sowing

Pests of other pasture legume species should be controlled, particularly in the first few weeks after sowing. Particular attention should be given to controlling redlegged earth mite, aphids, lucerne flea and caterpillars.

Grass selective herbicides are likely to be useful for controlling grassy weeds. Little is known at this stage about the most appropriate herbicides to use for post-emergent control of broadleaf weeds. Good pre-emergent weed control is a key strategy. This is best done by commencing the year before sowing, by reducing seed set of annual weeds in spring, and killing germinating weeds seedlings at the break of season before sowing.

8. Defer grazing until plants are well-rooted

Optimum grazing management strategies for tedera have yet to be devised. However, grazing should not commence until seedlings are firmly anchored and cannot be readily pulled up by hand. This is particularly important for a perennial species like tedera, as the opportunities to recruit new seedlings from seed set are likely to be limited, so long-term plant density will be related to the density of plants that successfully establish.

This package can only be regarded as preliminary, but it provides a framework for agronomic studies to further refine optimum establishment conditions for tedera.

5 Success in Achieving Objectives

5.1 Development of establishment packages

5.1.1 Sub-tropical grasses

The project was successful in developing a cost-effective establishment package for reliable establishment of exotic warm-season perennial grasses, including Rhodes grass (*Chloris gayana*), panic grass (*Megathyrsus maximum*), signal grass (*Urochloa brachiaria*), setaria (*Setaria spp.*), digit grass (*Digitaria eriantha*), Bambatsi panic (*Panicum coloratum*) and kikuyu (*Pennisetum clandestinum*). This is a mature package that has been widely adopted by farmers and seeding contractors in south-western Australia, and is being promoted by the Evergreen group and catchment councils. Greater confidence in achieving establishment success has been a key driver in increasing the area sown to sub-tropical grasses in WA from an estimated 120,000 ha at the commencement of the project to a current area of 150,000 ha (P. Sanford, unpublished data).

5.1.2 Old man saltbush

The project was successful in developing a direct seeding package for old man saltbush (*Atriplex nummularia*), using conventional farm seeding equipment. It can be used for revegetating slightly- to moderately-saline land (EC_e values at 0-30 cm depth between 2 and 8 dS/m) and to rangeland environments. This technology now provides landholders with a reliable, low-cost method of establishing old man saltbush. Costs are expected to be around ($100-150/ha), compared to the previously more reliable, but more expensive method of transplanting nursery-raised seedlings of more than $450/ha.

5.1.3 Mallee saltbush (*Rhagodia preissii*)

The direst seeding package developed for old man saltbush also appears appropriate for direct seeding of *Rhagodia preissii*. However, this is based on the success of a single trial and further trials are required to demonstrate repeatability of establishment in different districts and on other soil types.

5.1.4 Tedera

This project was successful in developing a preliminary establishment package for tedera. The key finding from this research is that there do not appear to be any major barriers to successful germination and establishment of tedera in the field, provided soil moisture conditions are adequate. An establishment package for tedera has been developed, on the basis of experimental results from this project and from common agronomic practices used for other crops and pastures. The package provides a framework for agronomic studies to further refine optimum establishment conditions for tedera.

5.1.5 Establishment of native grasses

This project has produced a greater understanding of the germination biology of native grass seeds, which has allowed significant improvements in germination performance. The use of dormancy breaking treatments and germination stimulants has further improved germination performance of several species of native grass seed in laboratory environments.

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species tested. The approach used in this project can be readily adapted to industry to improve the quality of the end product. Further agronomic research is needed, however, to test these treatments in the field to maximise their potential.

5.1.6 Other species

Some species originally on the list for evaluation were found to have few barriers to germination and establishment. This included puccinellia (*Puccinellia ciliata*), tall wheat grass (*Thinopyrum ponticum*), *Lotus tenuis*, *L. australis* and lucerne (*Medicago sativa*) and no further work was done with them.

Other species require further work to better understand their seed biology and agronomic requirements before reliable establishment packages can be developed. This includes several of the chenopod species, such as river saltbush (*Atriplex amnicola*), wavy leaf saltbush (*A. undulata*), creeping saltbush (*A. semibaccata*), silver saltbush (*A. bunburyana*), small leaved blue bush (*Maireana brevifolia*) and sago bush (*M. pyramidata*).

5.2 Development of strategies for enhancing recruitment of native grasses

This project was successful in developing a simple, low-cost pasture management package to increase recruitment of desirable perennial grass species in native pastures.

Management guidelines were developed to increase the content of wallaby grass (*Austrodanthonia* spp.) and red grass (*Bothriochloa macra*), two important native grasses in the central tablelands of NSW, but should also be applicable to other native grasses in the high rainfall zone throughout south-eastern Australia. The principles can also be applied to seedling recruitment in sown perennial grass pastures.

5.3 Increased understanding of seed biology

This project resulted in a marked increase in our knowledge of the underlying seed biology of the species studied in this project. This increased understanding was a key factor in developing successful establishment and seedling recruitment packages.

An understanding of the temperature requirements for germination led to defining the “sowing window”, for different species in different regions. This provided a much better understanding of the optimum sowing times for each species. For example, it showed that old man saltbush and most of the other saltbushes could be sown in most districts of WA in August-September. It also showed that the requirement of temperatures > 30^{o}C for germination of small leaf bluebush precluded it from being considered for direct seeding in much of southern Australia.

Germination studies highlighted poor seed quality as a major constraint to successful germination and establishment of many perennial species. Post-harvest seed dormancy was identified in some species. This led to recommendations not to sow seed harvested earlier in the year of panic grass, signal grass or setaria, but to store it and use it the following year to maximise seed germination.

The use of dormancy breaking treatments and germination stimulants, such as smoke water, gibberellic acid, heat treatment and manipulation of florets, improved germination in the laboratory of some species from 5% to more than 90% in some cases. This was particularly important for improving the germination of several native grass species, including *Austrodanthonia* species, *Chloris truncata*, *Themeda triandra*, *Dichanthium sericeum*, *Enteropogon acicularis* and *Microlaena* *stipoides*. This quality increase can potentially facilitate the broad scale adoption of native grasses in Australia. However, the translation of these treatments to enhanced establishment in the field requires further research.

5.4 Development of specialised seeding machinery for saltland and rangeland species

An original aim of the project was to provide information that a commercial company could use to develop an improved niche seeder for sowing saltland and rangeland pasture species. However, the early success of direct seeding old man saltbush with an experimental cone-seeder, led us to change this aim to develop direct seeding technology for use with conventional farm machinery, to encourage farmers to sow seed with their own seeding machinery. This was seen as a more effective way to increase adoption of old man saltbush over large saltland and rangeland areas.

This change in direction was made in consultation with MLA, AWI and the FFI CRC.

There is now considerable interest by a group of leading saltland farmers and saltbush seeding contractors in developing this concept further for sowing large areas of saltbush with their own machinery.

5.5 Publications

An initial project objective, stated in the project submission, was "*to publish two extension products and at least five scientific papers".* The extension products were to comprise:

a)." A Technical Bulletin, that provides a comprehensive overview of the germination and establishment knowledge of the target species gained in the project (Target audience: Extension Officers, NRM Officers, Consultants, Policy Makers, Catchment Management Authorities, Seed Producers, Seed Merchants, Seeding Contractors, Funding Providers, Program Managers, Students); and

b). Practical and farmer-friendly publications that provide specific establishment recipes for individual species and regions, such as Farmnotes/Agnotes/Agfacts series and other extension publications (Target audience: Farmers, Pastoralists, Extension Officers, Consultants, Seed Merchants, Seeding Contractors, Catchment Management Authorities)".

The six sub-projects were conducted over a disparate range of plant groups, suited to a range of different farming systems. In compiling the large set of results from the different sub-projects, it became apparent that individual Technical bulletins, each focussing on a different group of material, would be a much more manageable and appropriate way of getting the messages from the project out to the various interest groups, than a single large Technical Bulletin that covered all project outputs. Consequently, several smaller, focussed Technical and Advisory bulletins have been written.

Five Technical bulletins, two Extension bulletins and three Advisory notes have been prepared for publication. These are targeted at the different audiences, depending on the level and detail of technical detail required. These have been attached as Attachments to this report.

5.5.1 Technical bulletins

Five Technical bulletins have been prepared for publication. These publications are highly technical scientific publications containing details of the experimental results and conclusions used to formulate each of the establishment packages. These will be published as FFI CRC Technical bulletins, with the main audience being other Scientists, Extension officers, Consultants, Funding providers and tertiary students.

The Technical bulletins prepared for publication are:

- "*Establishment of sub-tropical grasses in south-western Australia*" by P.G.H. Nichols, R.J. Yates, C. Loo, B. Wintle, J.W. Titterington, E.G. Barrett-Lennard, J. C. Stevens, K.W. Dixon and G.A. Moore (Attachment 1)
- "*Direct seeding of chenopod shrubs for saltland and rangeland environments*" by P.G.H. Nichols, R.J. Yates, C. Loo, J.C Stevens, J. W. Titterington, B.J. Wintle, G.A. Moore, K.W. Dixon and E.G. Barrett-Lennard (Attachment 2)
- "*Establishment of native perennial grasses*" by J.S. Stevens, S. Clarke, M. Mitchell, M.H. Ryan, I. Chivers, C. Loo, K.W. Dixon. and P.G.H. Nichols (Attachment 3)
- "*Low Cost Rehabilitation of Native Perennial Grass Pastures by Managing Seedling Recruitment*" by R. Thapa, D.W. Kemp and P.G.H. Nichols (Attachment 4)
- "*Establishment of tedera (Bituminaria bituminosa var. albo-marginata)*" by P.G.H. Nichols, C. Beard, C. Loo and P. Michael (Attachment 5)

5.5.2 Extension bulletins

These publications are practically orientated. They contain background information on the establishment packages and provide some science to the recommendations. Their main audience is leading farmers, Extension Officers, NRM Officers, Consultants, Policy Makers, Catchment Management Authorities, Seed Producers, Seed Merchants, Seeding Contractors, Funding Providers and Program Managers.

One Extension bulletin has been published on identifying sub-tropical grass seedlings. This shows photographs and gives descriptions of seedlings of Rhodes grass, panic grass, signal grass, digit grass, setaria, kikuyu and Bambatsi panic grass. This has been published and distributed to 600 subscribers of the Evergreen newsletter.

Two other Extension bulletins have been prepared for publication. The sub-tropical grass Extension bulletin contains background information on the establishment package and some farmer case studies.

These Extension bulletins are:

- "*Identifying sub-tropical grass seedlings*" by Brad Wintle, Geoff Moore and Phil Nichols (2009), Bulletin No 4775, Department of Agriculture and Food Western Australia, South Perth (Attachment 6).
- "*Establishment guide for sub-tropical grasses – key steps to success*" by Geoff Moore, Ron Yates, Phil Nichols, Brad Wintle and John Titterington, Phil Barrett-Lennard and Chris Loo (Attachment 7).

5.5.3 Advisory notes

One Advisory note has been published on establishing sub-tropical grasses and has been distributed to 600 subscribers of the Evergreen newsletter. Two other Advisory notes have been prepared for publication. These publications provide succinct information on the steps for successful plant establishment.

These Advisory notes are:

- *"Establishing sub-tropical perennial grasses*" by Phil Nichols, Ron Yates and Geoff Moore (2010), Department of Agriculture and Food Western Australia, Farmnote No 443. (Attachment 8)
- "Direct seeding of old man saltbush" by Phil Nichols, Ron Yates and Ed Barrett-Lennard (Attachment 9)
- "Recruitment of desirable perennial grass seedlings within existing pastures" by Roshan Thapa and David Kemp (Attachment 10)

5.5.4 Scientific publications

Six scientific papers have been published, or have been submitted for publication in the journal *Crop and Pasture Science* (formerly the *Australian Journal of Agricultural Research*), while three other are papers are under preparation.

These papers submitted for publication are:

- Stevens, J.C., Barrett-Lennard, E.G. and Dixon, K.W. (2006). Enhancing the germination of three fodder shrubs (*Atriplex anicola*, *A, nummularia*, *A. undulata*; Chenopodiaceae): implications for the optimisation of field establishment. *Australian Journal of Agricultural Research* **57**: 1279-1298. (Attachment 11)
- Thapa, R., Kemp, D.R., Michalk, D.L. and Simmons, A.T. (2011). Effects of biomass manipulation, seed level modification, and pasture composition on recruitment of *Austradanthonia* spp. in existing swards. *Crop and Pasture Science* (submitted) (Attachment 12)
- Thapa, R., Kemp, D.R., Michalk, D.L. and Badgery, W.B. (2011). Effects of biomass manipulation, seed level modification, and pasture composition on recruitment of *Bothriocloa macra*. in existing swards. *Crop and Pasture Science* (submitted) (Attachment 13)
- Thapa, R., Kemp, D.R. and Mitchell, M.L. (2011). Suitable climatic events for a seedling recruitment event within existing perennial grass swards in south-eastern Australia. *Crop and Pasture Science* (submitted) (Attachment 14)
- Thapa, R., Kemp, and D.R. Michalk, D.L. (2011). Recruitment of *Phalaris aquatica* within existing swards. 1. Effects of biomass manipulation, seed level modification, and site preparation. *Crop and Pasture Science* (submitted) (Attachment 15)
- Thapa, R., Kemp, and D.R. Michalk, D.L. (2011). Recruitment of *Phalaris aquatica* within existing swards. 2. Effects of pasture composition. *Crop and Pasture Science* (submitted) (Attachment 16)

Three other papers outlining different aspects of the establishment packages are under preparation for submission to *Crop and Pasture Science*. These are:

- R.J. Yates, C. Loo, G.A. Moore and P.G.H. Nichols, Influence of depth and time of sowing on the establishment of warm season perennial pastures in the agricultural regions of Western Australia. (Attachment 17)
- C. Loo, J.C. Stevens, K.W. Dixon, R.J. Yates, E.G. Barrett-Lennard, G.A. Moore and P.G.H. Nichols, Plant signalling chemicals for enhanced germination and emergence in perennial pasture species. Attachment 18)

- P.G.H. Nichols, R.J. Yates, E.G. Barrett-Lennard and C. Loo, Direct seeding of old man saltbush. Attachment 19)

6 Impact on the Meat Industry

6.1 Impact now

6.1.1 Increase in adoption and improved establishment of sub-tropical perennial grasses

Adoption of the establishment package for sub-tropical perennial grasses has been rapid, with farmers now having much greater confidence in being able to achieve satisfactory establishment densities. Mean establishment densities on commercial farms have increased ten-fold from original densities in the order of 1 plant/m^2. This has lead to greater confidence in sub-tropical grasses, with the estimated area sown in south-western WA increasing from 120,000 ha to 150,000 ha since commencement of the project (P. Sanford, unpublished data). This means farmers are already benefiting from increased farm productivity and provision of out-of-season feed provided by the perennials, resulting in increased carrying capacity. More reliable establishment means that short-medium term subtropical perennial grass pastures will be more productive, due to increased density of the sown grasses. An indirect impact has been an increase in the price of land established to perennial pastures, as most paddocks in the main target zone consist of sands that are marginal or unsuitable for cropping and support poor annual pastures.

Rapid adoption of the establishment package has been due in a large way to its simplicity and practicality and by educating farmers to give them a much greater understanding of the key factors for success. The fact they do not have to buy specialised seeding equipment, but can make modifications to their own machinery, has made the package attractive to farmers. The close interaction of the project with producers and seeding contractors in the Evergreen group, the Northern Agricultural Catchment Council (NACC) and the Mingenew-Irwin and West Midlands farmer groups, in particular, has also been a major factor in rapid adoption, as these groups have provided the extension conduits for key messages from the project. A series of field days and workshops have been held with each of these groups and articles have been written for each of their newsletters. The farmnote *"Establishing sub-tropical perennial grasses*" has also been mailed out directly to the 600 members of the Evergreen group.

6.1.2 Technology transfer of the direct seeding package for old man saltbush

The direct seeding package for old man saltbush now provides landholders with a reliable, low-cost method of establishing old man saltbush. Costs are expected to be around ($100-150/ha), compared to the previously more reliable, but more expensive method (more than $450/ha), of transplanting nursery-raised seedlings.

The project has shown that it is feasible for old man saltbush to be sown using conventional farm seeding equipment, with some modifications for wide row spacings and depth control. This seeding technology is now ready to be taken to a commercial scale. Several farmers and saltbush seeding contractors are intending to develop this concept further for sowing large areas of saltbush with their own machinery. This will allow the project findings to be implemented on a wider scale. This is likely to lead to substantial farmer and grazier adoption in the longer term. Furthermore, there is a high chance that the developed techniques would enable direct seeding in the rangelands and arid areas of the wheatbelt.

A key finding of the project was subspecies and genotypic differences within old man saltbush for ability to establish from direct seeding. Subspecies *nummularia* was found to be suited to direct seeding, but genotypic differences were found within this subspecies for expression of this trait; seed of types that readily established from seed (Emergent types) have been included in the FFI CRC saltbush breeding program. On the other hand, subsp. *spathulata* was found to be unsuited to direct sowing; this subspecies has also been found to be unpalatable to grazing sheep in other FFI CRC studies. Farmers, seeding contractors and seed merchants now have the information to ensure that subsp. *nummularia* is planted in preference to the less desirable subsp. *spathulata*.

6.1.3 Use of seed treatments for sowing native grasses

The use of dormancy breaking treatments and germination stimulants, such as smoke water, gibberellic acid, heat treatment and manipulation of florets has been shown to help break down post-harvest seed dormancy and improve germination of several native grasses under laboratory conditions. However, translation of these results to the field has been problematic and further research is required. Until this is achieved, native grasses are likely to remain in the domain of rehabilitation programs, where high establishment costs are not a major concern.

6.1.4 Management to increase recruitment of native grasses

The research presented in this project suggests that a steady improvement in the perennial grass content of average pastures across south-eastern Australia is now possible to restore native pastures to a more productive state. Previous research in the NSW Tablelands indicated that at least 60% perennial grass is needed to have useful impacts on livestock production, weed control, lessen acid soil development and better water use to minimise salinity problems (Michalk *et al.* 2003). However, surveys found that the perennial grass content of pastures in this area was often less than 20% (Kemp and Dowling, 1991). Farmers now have a low-cost approach to pasture improvement for those large parts of the landscape where there are still some desirable perennial grasses and no over-riding weed problems. The restored pasture may not be as productive as a newly sown pasture, but the investment costs are considerably less and hence restored paddocks could prove more profitable.

A conservative rate of improvement in perennial grass content may only be 10% *p.a.* (of the total herbage mass), such that from an average of 20%, it could take 4 years to reach a target of 60%. Costs involved could vary from nil (e.g. when resting the paddock) to $20/ha for a light scarification to create micro-sites to improve recruitment. This is in contrast to $300/ha to sow a new pasture. Through the period of fostering recruitment to bringing the perennial grass content to 60% the carrying capacity of a paddock may not change initially. But once the target is reached then either more stock or more production per head will result. On many of the poor native grass areas in NSW it is not unreasonable to anticipate doubling production in the medium term.

6.1.5 Confirmation that tedera has no major barriers to establishment

Tedera is being extensively researched by the Future Farm Industries CRC as a new drought tolerant perennial pasture legume and a commercial release is not likely for at least five years. However, this project has developed a preliminary establishment package for the species and demonstrated that there are no major barriers to its establishment in the field. This will have immediate benefits for researchers establishing field trials of tedera and will be applicable to growers once it is commercialised.

6.2 Impact in five years time

6.2.1 Further adoption of sub-tropical grasses

In five years time the area of adoption of sub-tropical perennial grasses is likely to further increase, due to increased confidence by growers in their ease and reliability of establishment. The main target area in Western Australia will continue to be sands that are marginal or unsuitable for cropping and to coastal areas subject to periodic waterlogging. However, an expansion into lower rainfall areas and different soil types, particularly in the northern agricultural area, is likely as a form of risk management against climate change, particularly if returns from livestock production increase.

Farmers will enjoy both productivity and environmental benefits from better sub-tropical perennial pastures, attributable to greater establishment densities. Overall carrying capacity will increase, due to provision of more out of season green feed, improved seasonal distribution of feed and reduced reliance on supplementary feed and conserved fodder in autumn. The environment will benefit from increased water use and reduced groundwater recharge (with reductions in the potential for dryland salinity) and reduced wind erosion potential in summer (Moore *et al.* 2006). Land established to sub-tropical perennial grasses will continue to have higher dollar value than those without.

6.2.2 Widespread sowing of old man saltbush

In five years time, leading farmers and seeding contractors will have taken on board the findings of this project to make small modifications to their conventional seeding machinery to direct seed old man saltbush. This is likely to lead to substantially increased adoption of old man saltbush, with large-scale plantings in new areas, including the rangelands and arid areas of the wheatbelt.

This technology will be attractive to landholders, as they will be able to establish old man saltbush reliably at a third of the cost of transplanting nursery-raised seedlings. It is also likely to be readily adopted, due to its simplicity and practicality and the fact that farmers will be able to sow stands of old man saltbush using their own machinery.

In five years time, new cultivars old man saltbush bred for ease of direct sowing, will be under development. Further improvements in establishment success will follow if the direct seeding technology is combined with “direct seeding-ready” cultivars of old man saltbush.

6.2.3 Increased pasture content of desirable native grasses

Native grasses will be increasingly seen as desirable in pastures in the high rainfall, non-arable regions of south-eastern Australia, due to their productivity benefits and from increasing pressure by environmentalist to maintain plant biodiversity. This increase will come from use of the pasture management strategies developed in this project to increase recruitment of desirable native grasses within existing pastures, or from sowing native grass cultivars.

The pasture management strategies to increase native grass recruitment will become more mainstream across south-eastern Australia as farmers gain greater confidence in them and see improved pasture productivity benefits. If these strategies are followed, it is likely to take 4-5 years for paddocks with a current perennial grass content average of 20% to reach the desired content of 60% from. At this point increased livestock production will be clearly greater than for the current degraded state, either through increased carrying capacity or more production per head. On many of the poor native grass areas in NSW it is not unreasonable to anticipate doubling production in the medium term. This technology is likely to be attractive to graziers, due to its minimal input requirements and considerably lower costs (< $20/ha) than the $300/ha required to sow a new pasture. The restored pasture may not be as productive as a newly sown pasture, but the investment costs will be considerably less and hence, restored paddocks could prove more profitable.

In five years time, further understanding of the seed biology of important native grasses and further developments in seed priming and coating technologies will result in more reliable establishment technologies and decreased seeding costs. This will take the main area of use from rehabilitating small-scale degraded areas of the landscape to broadacre farm paddocks, for which the high establishment costs are prohibitive. This will lead to increased adoption of native grasses.

6.2.4 Sowing new cultivars of tedera

One or more cultivars of tedera will have been released as a new drought tolerant perennial pasture legume in five or so years time. The establishment package developed in this project will then be used by farmers to sow stands of this new species. The lack of major barriers to sowing and establishment suggests that adoption of tedera will be rapid, provided seed costs are not prohibitive. Farmers will then benefit from increased farm productivity and provision of out-of-season feed provided by this perennial. Widespread adoption will benefit the community through increased planting of a deep-rooted perennial that helps reduce rising water-tables and the impact of dryland salinity.

7 Conclusions and Recommendations

7.1 Development of establishment packages

This project was very successful in developing an establishment package for sub-tropical grasses. This is now a mature technology that has been taken up by many farmers. Little additional work is required to fine-tune this technology from the perennial grass perspective. The biggest need now is to develop methods to incorporate a legume component into sub-tropical grass pastures to provide nitrogen and maintain pasture productivity.

The project was very successful in developing establishment packages for old man saltbush, *Rhagodia preissii* and tedera. This seeding technology now needs to be taken to a commercial scale for further developmental work

The project was also highly successful in developing a package for farmers to increase the recruitment of desirable native perennial grasses within their existing pastures. This package needs an extension phase to test robustness of the recommendations and to promote the practice to farmers in different areas in south-eastern Australia.

The rapid uptake of the package for sub-tropical grasses shows that new agronomic practices can be readily adopted by farmers if they have the following features:

- the technology is simple and practical;
- they can use their own existing machinery (possibly with some modifications);
- it is inexpensive; and
- the benefits from using the technology are obvious.

The successful establishment packages developed for the other species share these traits and are, therefore, likely to be adopted if they are promoted widely enough.

7.2 Species requiring further work

7.2.1 Other chenopod species

This project was not able to develop reliable establishment packages for other *Atriplex* species including *A. amnicola* and *A. undulata*. The project had limited success with these species, which presently work well with the "Mallen" niche seeder. Further work is needed to understand the triggers for seedling emergence before these species can be direct-seeded with conventional machinery.

Direct sowing of bluebushes (*Maeriana brevifolia* and *M. pyramidata*) appears to be problematic in much of southern Australia. The main difficulty is their requirement for warm temperatures (~30^{o}C) to germinate. These temperatures do not normally occur until November in southern Australia, by which time the winter growing season has generally ceased. Further work is required to understand the germination ecology of these species. Field observations suggest recruitment of new bluebush seedlings occurs from surrounding bushes after episodic summer rainfall events. This suggests an alternative and potentially cheap method of establishing bluebushes is to encourage natural recruitment of seedlings from seed produced on surrounding bushes (if present). This is a similar approach to that taken in Sub-program 5 of encouraging recruitment of native grasses and similar studies would be required to better understand this process. To introduce bluebushes into virgin sites a low density of nursery-raised seedlings could be transplanted to act as a seed source for natural recruitment. In order to devise such strategies there is clearly a need for studies to better understand their ecology and cues for germination. It may also be possible to identify populations or genotypes of both species with lower temperature requirements for germination, that make them more suited to direct sowing in late winter or early spring.

7.2.2 Other native grasses

The use of dormancy breaking treatments and germination stimulants, such as smoke water, gibberellic acid, heat treatment and manipulation of florets, was shown to help break down post-harvest seed dormancy and improve germination of several native grasses under laboratory conditions. However, further research is required to transfer the benefits of these results to the field. This needs to be combined with further agronomic experimentation. Until this is achieved, native grasses are likely to remain in the domain of rehabilitation programs, where high establishment costs are not a major concern.

7.3 Understanding the constraints to establishment and recruitment

The development of reliable establishment packages for warm season perennial grasses, old man saltbush and tedera will result in greater adoption of these species, due to increased confidence by growers in their ease and reliability of establishment.

This project benefited enormously by combining detailed laboratory studies on seed biology with agronomic studies in the field. In this way, constraints to establishment, such as seed dormancy, were able to be understood and methods to overcome them could be tested in the field. Laboratory studies to determine optimum temperatures for germination were able to be translated to optimum times for sowing in the field. In a similar way, detailed ecological measurements of germination events were able to be used to devise strategies to enhance recruitment of native grasses.

Some common themes for successful establishment emerged from the project across species. These included:

- Selecting paddocks a year ahead of sowing and reducing weed seed set

- Selecting species and varieties adapted to the district, soil type and intended use
- Using good quality seed
- Preparing a weed-free seed bed prior to sowing
- Sowing into a moist seedbed at the appropriate sowing time for the species
- Ensuring that seeding depth is carefully controlled, particularly for small-seeded species
- Using seeding machinery that sows into furrows, scalps non-wetting sand and weeds and uses press wheels
- Controlling weeds and pests (insects, kangaroos and rabbits) post-sowing
- Deferring grazing until plants are well established

7.4 Seed quality

Poor seed quality was identified as a major factor limiting establishment success in many of the perennial species studied. This means that even if all the recommended establishment steps are followed correctly, there is still the potential for a poor seeding result, simply because seed of low germinability was sown. Unlike crop species and the majority of exotic temperate pasture species, there are no seed certification schemes for warm-season perennials, saltbushes or native species and it is very much a case of “buyer beware”. An education program to make growers more aware of seed quality issues is likely to have the greatest effect on influencing the seed industry to increase seed quality standards for perennial pasture species. Some targeted research and development into seed production of important perennial species to improve seed quality and increase harvest yields (and thereby reduce seed costs) may be warranted.

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9 List of attachments

The following 19 attachments are appended as separate files.

Technical bulletins:

1. "*Establishment of sub-tropical grasses in south-western Australia*" by P.G.H. Nichols, R.J. Yates, C. Loo, B. Wintle, J.W. Titterington, E.G. Barrett-Lennard, J. C. Stevens, K.W. Dixon and G.A. Moore (Attachment 1)
2. "*Direct seeding of chenopod shrubs for saltland and rangeland environments*" by P.G.H. Nichols, R.J. Yates, C. Loo, J.C Stevens, J. W. Titterington, B.J. Wintle, G.A. Moore, K.W. Dixon and E.G. Barrett-Lennard (Attachment 2)
3. "*Establishment of native perennial grasses*" by J.S. Stevens, S. Clarke, M. Mitchell, M.H. Ryan, I. Chivers, C. Loo, K.W. Dixon. and P.G.H. Nichols (Attachment 3)
4. "*Low Cost Rehabilitation of Native Perennial Grass Pastures by Managing Seedling Recruitment*" by R. Thapa, D.W. Kemp and P.G.H. Nichols (Attachment 4)
5. "*Establishment of tedera (Bituminaria bituminosa var. albo-marginata)*" by P.G.H. Nichols, C. Beard, C. Loo and P. Michael (Attachment 5)

Extension bulletins

6. "*Identifying sub-tropical grass seedlings*" by Brad Wintle, Geoff Moore and Phil Nichols (2009), Bulletin No 4775, Department of Agriculture and Food Western Australia, South Perth (Attachment 6).
7. "*Establishment guide for sub-tropical grasses – key steps to success*" by Geoff Moore, Ron Yates, Phil Nichols, Brad Wintle and John Titterington, Phil Barrett-Lennard and Chris Loo (Attachment 7).

Advisory notes

8. "*Establishing sub-tropical perennial grasses*" by Phil Nichols, Ron Yates and Geoff Moore (2010), Department of Agriculture and Food Western Australia, Farmnote No 443. (Attachment 8)
9. "Direct seeding of old man saltbush" by Phil Nichols, Ron Yates and Ed Barrett-Lennard (Attachment 9)
10. "Recruitment of desirable perennial grass seedlings within existing pastures" by Roshan Thapa and David Kemp (Attachment 10)

Scientific publications

11. Stevens, J.C., Barrett-Lennard, E.G. and Dixon, K.W. (2006). Enhancing the germination of three fodder shrubs (*Atriplex anicola*, *A, nummularia*, *A. undulata*; Chenopodiaceae): implications for the optimisation of field establishment. *Australian Journal of Agricultural Research* **57**: 1279-1298. (Attachment 11)
12. Thapa, R., Kemp, D.R., Michalk, D.L. and Simmons, A.T. (2011). Effects of biomass manipulation, seed level modification, and pasture composition on recruitment of *Austrodanthonia* spp. in existing swards. *Crop and Pasture Science* (submitted) (Attachment 12)

13. Thapa, R., Kemp, D.R., Michalk, D.L. and Badgery, W.B. (2011). Effects of biomass manipulation, seed level modification, and pasture composition on recruitment of *Bothriocloa macra*. in existing swards. *Crop and Pasture Science* (submitted) (Attachment 13)
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15. Thapa, R., Kemp, and D.R. Michalk, D.L. (2011). Recruitment of *Phalaris aquatica* within existing swards. 1. Effects of biomass manipulation, seed level modification, and site preparation. *Crop and Pasture Science* (submitted) (Attachment 15)
16. Thapa, R., Kemp, and D.R. Michalk, D.L. (2011). Recruitment of *Phalaris aquatica* within existing swards. 2. Effects of pasture composition. *Crop and Pasture Science* (submitted) (Attachment 16)
17. R.J. Yates, C. Loo, G.A. Moore and P.G.H. Nichols, Influence of depth and time of sowing on the establishment of warm season perennial pastures in the agricultural regions of Western Australia. (Attachment 17)
18. C. Loo, J.C. Stevens, K.W. Dixon, R.J. Yates, E.G. Barrett-Lennard, G.A. Moore and P.G.H. Nichols, Plant signalling chemicals for enhanced germination and emergence in perennial pasture species. (Attachment 18)
19. P.G.H. Nichols, R.J. Yates, E.G. Barrett-Lennard and C. Loo, Direct seeding of old man saltbush. (Attachment 19)

Future Farm Industries CRC Technical Bulletin

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Abstract

Sub-tropical grasses are showing excellent potential in the Northern Agricultural Region (NAR) of Western Australia in areas with mild winters and where the rainfall is greater than 300 mm. They have also been widely used on the south coast of WA, where kikuyu, in particular, has been sown over an estimated area of 60–90,000 ha.

Four years ago, seeding failures of warm-season perennial grasses in Western Australia were common, with patchy establishment and densities of less than 1 plant/m^2. Greater understanding of their seed biology and agronomic requirements has led to the development of a reliable establishment package for warm-season perennial grasses. Rapid adoption of the key elements of the package has resulted in the bar being raised considerably throughout the industry and farmers now expect a much higher and even more plant establishment.

The ten key elements of the package are:

1. Plan a year ahead and reduce weed seed-set, commence control of rabbits and kangaroos and consider sowing a cereal to provide stubble for reduced erosion risk
2. Purchase good quality seed of appropriate species and varieties
3. Control weeds and insects prior to sowing
4. Sow into moisture in late winter - early spring (depending on district) - if soil moisture is limiting defer sowing until the following year
5. Set up the seeder to sow into furrows with trailing press wheels and a row spacing of 50-60 cm
6. Sow 2-5 kg/ha of seed, depending on seed quality and whether coated or uncoated
7. Sow at a depth of 5-10 mm
8. Don't sow too fast
9. Control weeds and pests (insects, kangaroos and rabbits) post-sowing
10. Defer grazing until grasses are well established

This bulletin provides information that provides an understanding of the key factors for successful establishment of sub-tropical perennial grasses. It is primarily aimed at farmers in south-western Australia, but the principles are also applicable to other areas with similar climates and soils.

Contents

1. Background

Sub-tropical perennial grasses in Australia have traditionally been grown in northern New South Wales and Queensland. However, they are now also being widely used in both the Northern Agricultural Region (NAR) and south coast areas of Western Australia. Kikuyu (*Pennisetum clandestinum*) has been grown on the south coast and Swan Coastal Plain for many years, while sowings of Rhodes grass (*Chloris gayana*), panic grass (*Megaththyrsus maximus* syn. *Panicum maximum*), signal grass (*Urchloa brachiaria*), setaria (*Setaria sphacelata* and *S. splendida*), and to a lesser extent digit grass (*Digitaria eriantha*) and bambatsi panic (*Panicum coloratum*), have increased in the past 10-15 years (Moore *et al.* 2006).

Productivity benefits from use of sub-tropical grasses include increased carrying capacity, due to provision of out of season green feed, improved seasonal distribution of feed and reduced reliance on supplementary feed and conserved fodder in autumn, while environmental benefits include increase water use and reduced drainage to groundwater (with reductions in the potential for dryland salinity) and reduced wind erosion potential in summer (Moore *et al.* 2006). Sub-tropical grasses can be grown on a wide range of soils, although their main use in Western Australia has been on sands that are marginal or unsuitable for cropping and to coastal areas subject to periodic waterlogging. On these soils, well managed perennial-based pastures can significantly increase production compared with annual volunteer pastures, in addition to their environmental benefits.

In the traditional areas of northern New South Wales and Queensland sub-tropical grasses are best sown in November- December, when soil temperatures are warm and there is a strong likelihood of summer rainfall (Lodge and Harden 2009). However, sowing at this time is generally not an option in south-western Australia, with its Mediterranean-type climate of wet winters and hot, dry summers. Consequently, establishment has often proved to be problematic in south-western Australia. Seeding failures have been common, with densities often less than 1 plant/m^2, while in other cases establishment has been inconsistent across paddocks, with a mixture of good and poor patches of established seedlings. However, the regular success by a small group of farmers indicated that successful establishment of sub-tropical perennial grasses was possible, although the factors resulting in success were not well understood.

This led to initiation of a research project in 2006 to gain a greater understanding of the seed biology and agronomic requirements for germination and establishment of warm-season perennial grasses, in order to develop a reliable establishment package for farmers and seeding contractors in south-western Australia. This project and its linkage to activities by the Evergreen Farming Group has led to rapid adoption of key elements of the package. This has resulted in the 'bar' being raised considerably throughout the industry and farmers now expect a much higher and more even establishment following sowing of sub-tropical grasses.

This bulletin describes a series of experiments aimed at determining the key factors affecting establishment of sub-tropical grasses in the NAR and south coast regions of Western Australia and uses this and information from other research programs and farmer experiences to present a package of the key steps for reliable establishment. The species Rhodes grass, panic grass, kikuyu, signal grass, setaria, digit grass and bambatsi panic are considered. Although this bulletin is primarily aimed at farmers in south-western Australia, the establishment package is also applicable to other areas of Australia and internationally with winter-dominant rainfall (Mediterranean-type) climates that are not prone to frosts.

2. Project details

A four-year project, titled "Reliable establishment of non-traditional perennial pasture species", commenced on 1 July 2006 as part of the former Cooperative Research Centre for Plant-based Management of Dryland Salinity (Salinity CRC), with industry funding from Meat & Livestock Australia, Australian Wool Innovation and the former Land and Water Australia. Management of the project was continued by the Future Farm Industries Cooperative Research Centre (FFI CRC), upon its inception in July 2007. The project consisted of four sub-projects: (i) "Establishment of exotic warm-season grasses and legumes"; (ii) "Establishment of saltland and rangeland species"; (iii) "Establishment of native grasses and legumes"; and (iv) "Recruitment of native grasses in native pastures". This bulletin focuses on results from the sub-project "Establishment of exotic warm-season grasses and legumes".

The warm-season grasses and legumes component of the project consisted of interlinked laboratory and field components. Kings Park BGPA conducted laboratory and glasshouse studies to gain an understanding of the seed biology and germination requirements of the species and also examined a range of potential germination enhancing seed treatments. DAFWA used this information to develop agronomic and seeding machinery solutions to develop agronomic packages for establishment.

2.1. Species and varieties

Selecting adapted species and cultivars for the soil type and climate of the sowing location is the first step to establishing a productive and persistent sub-tropical grass pasture. Sub-tropical grasses, apart from kikuyu, are very sensitive to frost. Their general area of adaptation in south-western Australia is approximately bounded by the 18^{o}C minimum temperature isohyet in August (Figure 1).

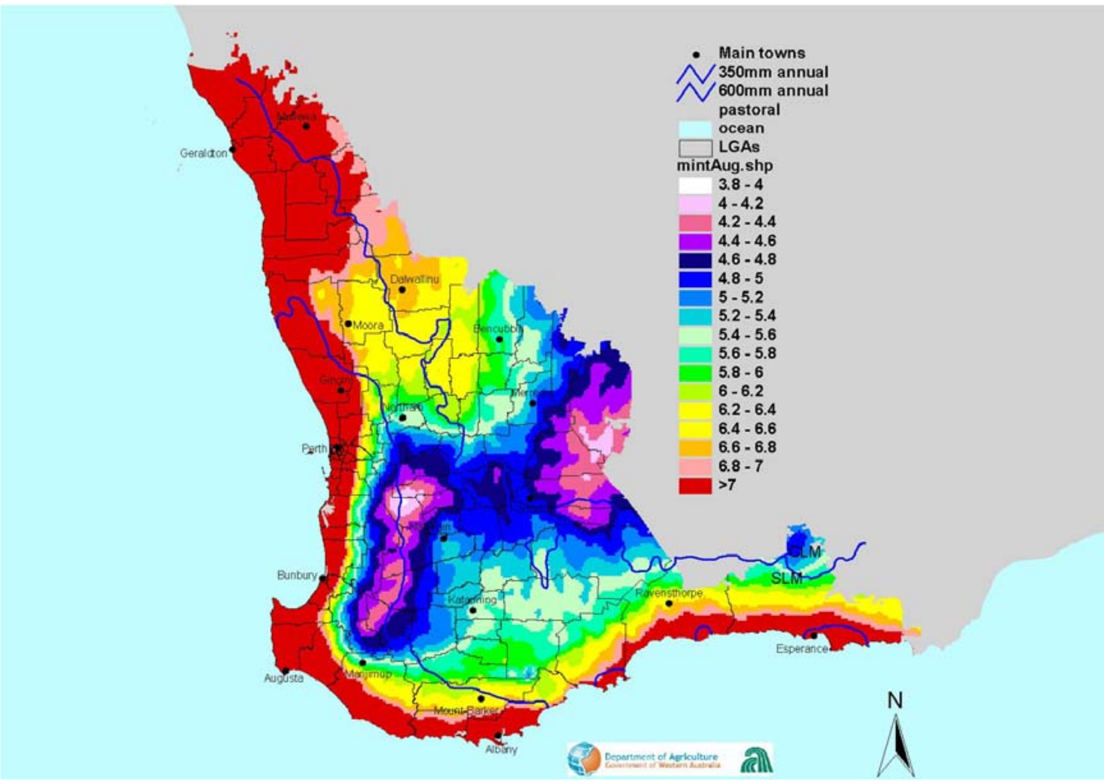


Figure 1. Map of south-western Australia showing August mean minimum temperatures (^{o}C). The adaptation zone of sub-tropical grasses is approximated by the areas shaded in red (taken from Moore *et al.* (2006)).

Figure 2. Seed of the sub-tropical grass species used in south-western Australia

Table 1. Sub-tropical grass species sown in south-western Australia, showing their soil and climatic suitability to the northern agricultural region (NAR) and south coast of Western Australia (adapted from Moore *et al.* (2006)).

Common name	**Species**	**Drought tolerance**	**Frost tolerance**	**Soil type**	**Soil pH_{Ca}**	**Waterlogging tolerance**	**Suitability to south coast**	**Suitability to NAR**
Rhodes grass	*Chloris gayana*	Moderate to high	Low	Range including deep sands	>4.3	Moderate	Well adapted (>400 mm)	Well adapted (>425 mm)
Digit grass	*Digitaria eriantha*	Moderate to high	Low to moderate	Range except deep pale sands	>4.2	Low	Moderately adapted (>400 mm)	Moderately adapted (>450 mm)
Panic grass	*Megathyrsus maxima* (syn. *Panicum maximum*)	High to very high	Low	Range including pale deep sands	>4.3	Low	Well adapted (>350 mm)	Well adapted (>325 mm)
Bambatsi panic	*Panicum coloratum*	High to very high	Low	Fine-textured clays	>5.0	Moderate	Niche species (>325 mm)	Niche species (>375 mm)
Kikuyu	*Pennisetum clandestinum*	Moderate to high	Moderate (re-grows from rhizomes)	Range including deep sands	>4.0	Moderate	Well adapted (>400 mm)	Niche species (>500 mm)
Setaria	*Setaria sphacelata*	Moderate	Low to moderate	Range, especially duplex soils	>5.0	Good	Well adapted (>475 mm)	Niche species (>550 mm)
Signal grass	*Urochloa brachiaria*	Moderate to high	Sensitive	Sandy soils	>4.0	Low to moderate	Not suitable	Moderately adapted (>450 mm)

3. Optimum density of sub-tropical perennial grasses

Creeping grasses, such as kikuyu and Rhodes and signal grasses, spread by above ground stolons or below ground rhizomes and have the potential to recover from a poor establishment density after two or more seasons, albeit with a loss of potential production until a satisfactory density has been reached. However, the bunch grasses, such as panic grass, setaria, digit grass and Bambatsi panic grass, do not spread and have little opportunity for seedling recruitment. For these species, good establishment densities are crucial if these grasses are to make a major contribution to the pasture. The crowns of bunch grasses, however, increase in size over time. This has an effect of increasing the competition for moisture between adjacent bunch grasses and reduces the space and available moisture for winter annuals. So what is the ideal establishment density of bunch grasses to optimise herbage production, both on an annual and seasonal basis, and how does it change over time? Two experiments were sown to provide answers to these questions.

Materials and methods

The effect of plant density on biomass production, plant size and persistence of transplanted Gatton panic seedlings was investigated at Wellstead on the south coast (34°32’44”S, 118°30’17”E), and at Badgingarra Research Station in the NAR (30°20’34”S, 115°32’21”E). Sites were established at Wellstead on October 3, 2006 and at Badgingarra on August 22, 2007.

Seedlings were raised in Jiffy pots in a glasshouse at South Perth for four weeks, prior to transplanting to the field. Six different plant densities (0.5, 1, 2, 4, 8 and 16 plants/m^2) were established into 5 m x 5 m plots (Figures 3 and 4). Density treatments were replicated four times and arranged in a randomised block design.

Plant counts, dry weights and botanical composition were measured at regular intervals over a 3-year period. Measurements were taken on the central 3 m x 3 m area of the plot, to avoid buffer effects. Mowing was conducted to a height of 60 mm and mown material was removed from the site. Plot areas were not grazed throughout the trial period. The diameter of plant crowns was measured after mowing on four plants in the central portion of each plot. Two perpendicular measurements were made on each plant and the results were averaged.

Feed quality measurements were taken from the Wellstead site on January 16, 2008. These were analysed by FEEDTEST Laboratories in Victoria. It was hypothesised that the bigger plants in the lower density plots would have higher quality feed than plants that were more stressed in the higher density plantings.

The Wellstead site suffered severe inundation when the nearby dam overflowed onto three of the four replicates following 187 mm of rain over a week in late November – early December 2008. Most plants died in these plots and measurements were only conducted on the remaining replicate thereafter.

Figure 3. Establishment density trial at Wellstead. Photograph taken in February 2007, following establishment in October 2006. Note the stressed plants in the centre of the dense plot (16 plant/m^2) in the foreground compared to the less dense plots behind it.

Figure 4. Establishment density trial at Badgingarra. Photograph taken in June 2009, following establishment in August 2007.

Results

Badgingarra

Monthly rainfall data for Badgingarra is shown in Figure 5. Establishment conditions were favourable, with 147 mm of rainfall from transplanting on August until the end of 2007. Annual rainfall for 2008, 2009 and 2010 was 527 mm, 450 mm and 380 mm, respectively, with the majority falling from May to September in each year.

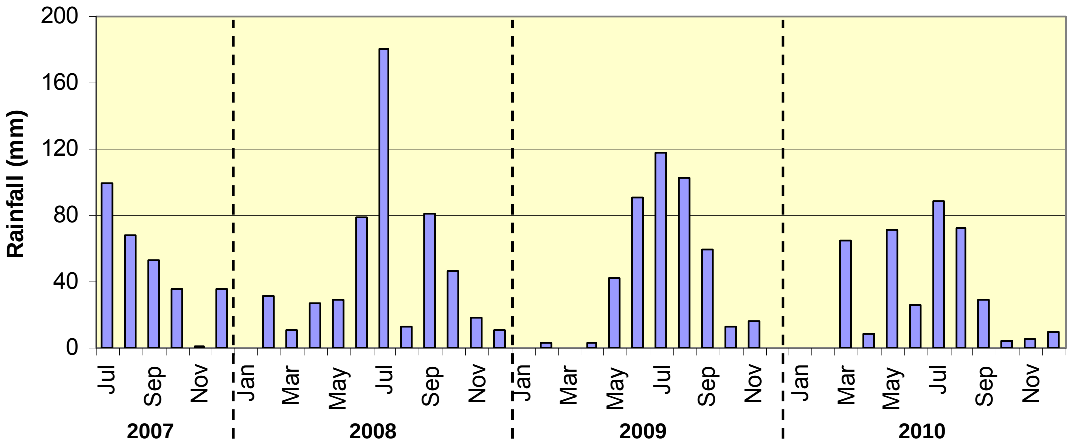


Figure 5. Monthly rainfall for Badgingarra Research Station from July 2007 to December 2010 (data from the Australian Bureau of Meteorology)

In December 2010 (three years after planting), all panic grass plants in the 0.5 and 1 plant/m^2 plots and more than 99% of plants in the other density treatments were still present. Crown diameter at this time was strongly negatively correlated with plant density ($r^2 = 0.94$, $P < 0.001$) (Figure 6).

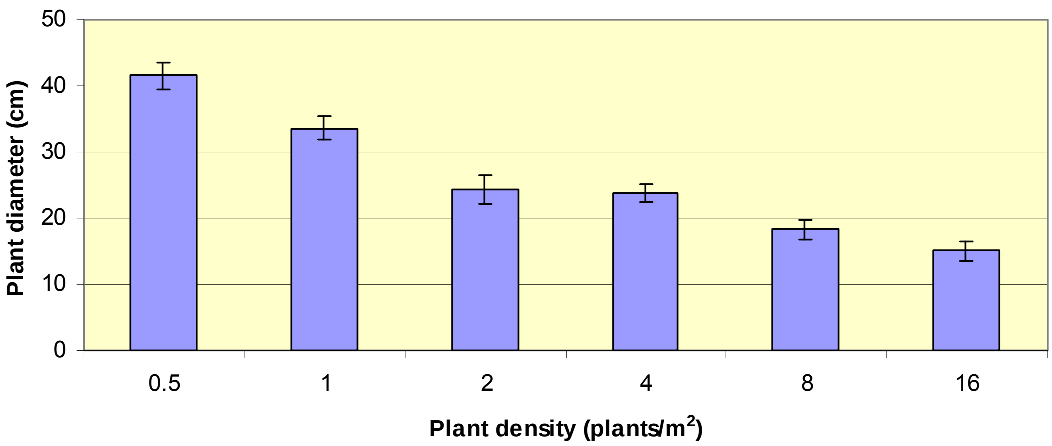


Figure 6. Crown diameter of panic grass plants in December 2010 when planted at densities of 0.5, 1, 2, 4, 8 or 16 plants/m^2 at Badgingarra (mean of 4 plants measured in 4 replicates)

Cumulative biomass of panic grass (three cuts) in the first year after planting is shown in Figure 7, while cumulative biomass of panic grass, annual legumes, grasses and herbs in the second and third years (total of four cuts in each) are shown in Figures 8 and 9, respectively.

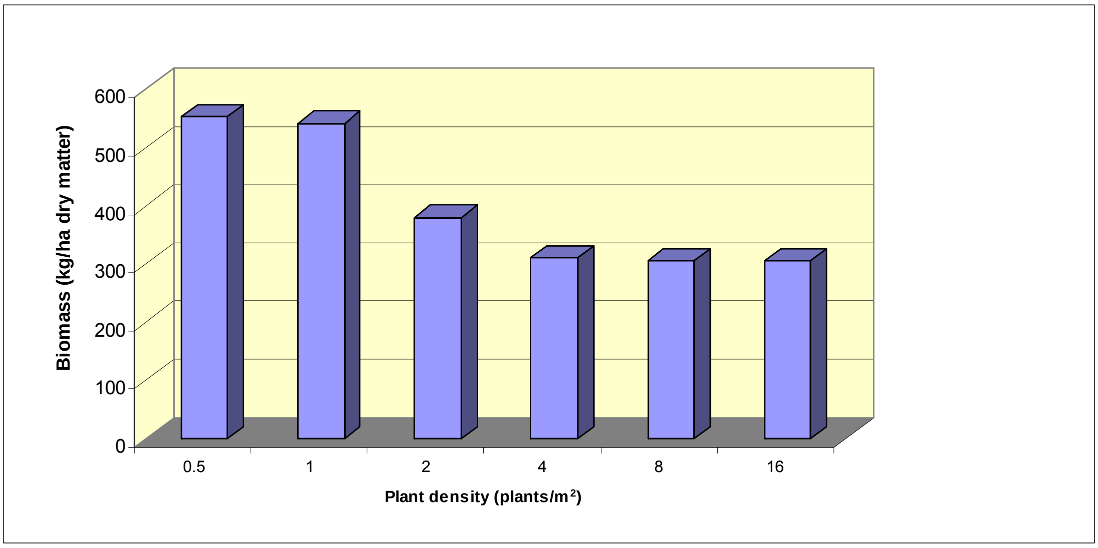


Figure 7. Cumulative panic grass biomass at Badgingarra from August 2007 to May 2008 (three cuts) when transplanted at densities of 0.5, 1, 2, 4, 8 or 16 plants/m^{2} (mean of 4 replicates)

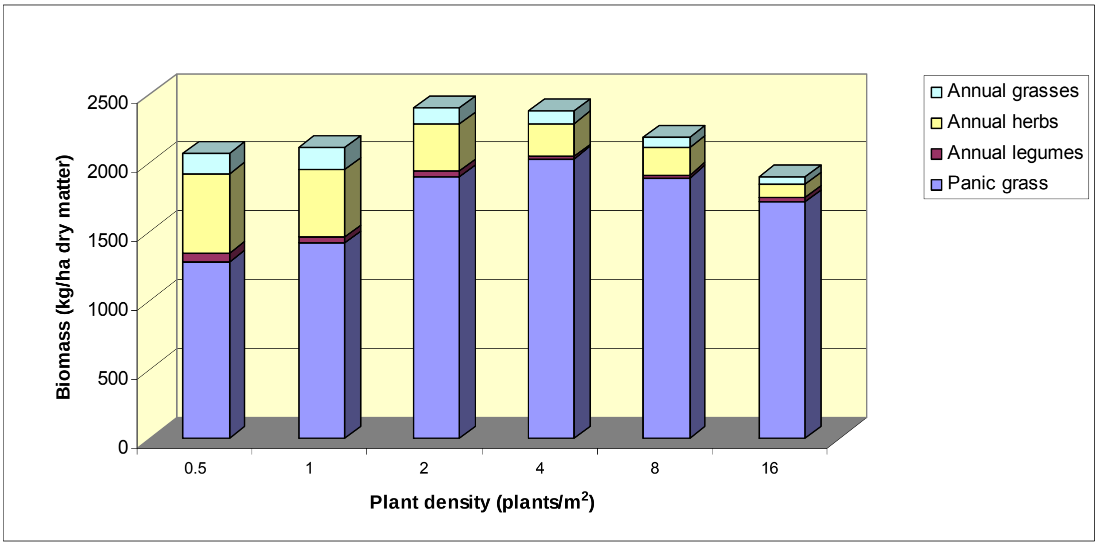


Figure 8. Cumulative biomass (total of four cuts) of panic grass, annual legumes, grasses and herbs in the second season (May 2008 to June 2009) after transplanting at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 at Badgingarra (mean of 4 replicates)

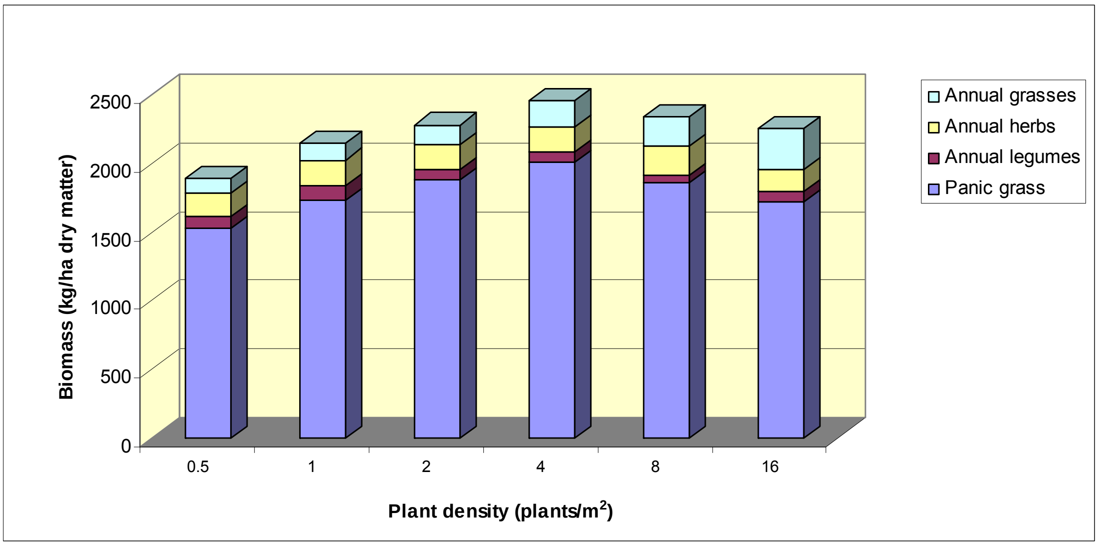


Figure 9. Cumulative biomass (total of four cuts) of panic grass, annual legumes, grasses and herbs in the third season (June 2009 to May 2010) after transplanting at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 at Badgingarra (mean of 4 replicates)

Wellstead

Monthly rainfall data for Wellstead is shown in Figure 10. Only 43 mm of rainfall fell from transplantation of seedlings in October to December 2006, while annual rainfall for 2007, 2008 and 2009 was 360 mm, 525 mm and 352 mm, respectively. Rainfall for January to June in 2010 was 180 mm.

More than 96% of all plants survived through to June 2008, with no differences in plant survival between plant density treatments. Most of these losses occurred over the first summer after planting. However, 187 mm of rain during late November – early December 2008 (see Figure 10) caused the nearby dam to overflow and inundate three of the four replicates, causing death of the majority of plants in these plots. Further measurements at this site were only conducted on the remaining replicate.

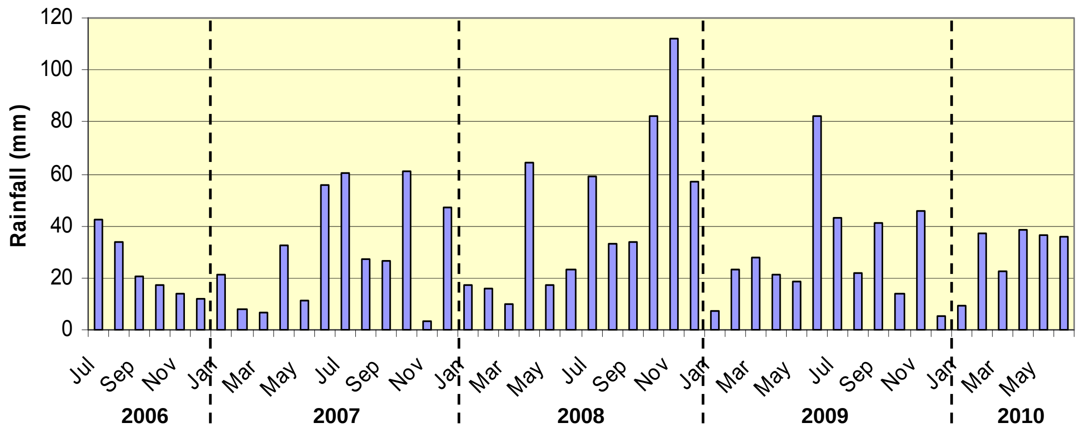


Figure 10. Monthly rainfall for Wellstead from July 2006 to July 2010 (data from the Department of Agriculture and Food Western Australia Client Resource and Information System)

In June 2010 (nearly four years after planting), more than 99% of all panic grass plants in each density treatment were still present in the remaining replicate. As was the case at Badgingarra, there was a strong negative relationship between crown diameter at this time with plant density (Figure 11).

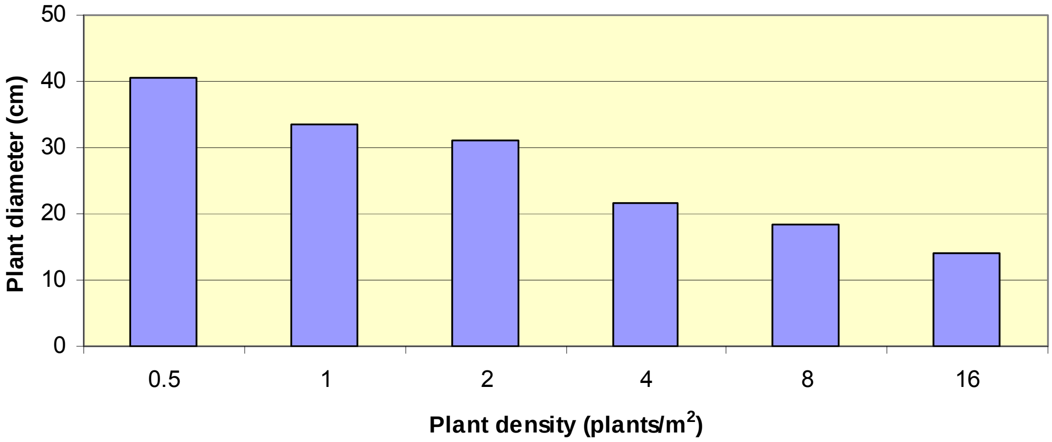


Figure 11. Plant diameter of panic grass plants in June 2010 when planted at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 at Wellstead. Results from 4 plants measured in only one replicate, due to destruction of the other three replicates.

Cumulative biomass of panic grass from October 2006 to June 2008 (seven cuts) is shown in Figure 12, while cumulative biomass of panic grass, annual legumes, grasses and herbs from June to October in 2008 (single cut) is shown in Figure 13. Cumulative biomass (total of six cuts) of panic grass, annual legumes, grasses and herbs from Feb 2009 to June 2010 is shown in Figure 14 (results from only one replicate only).

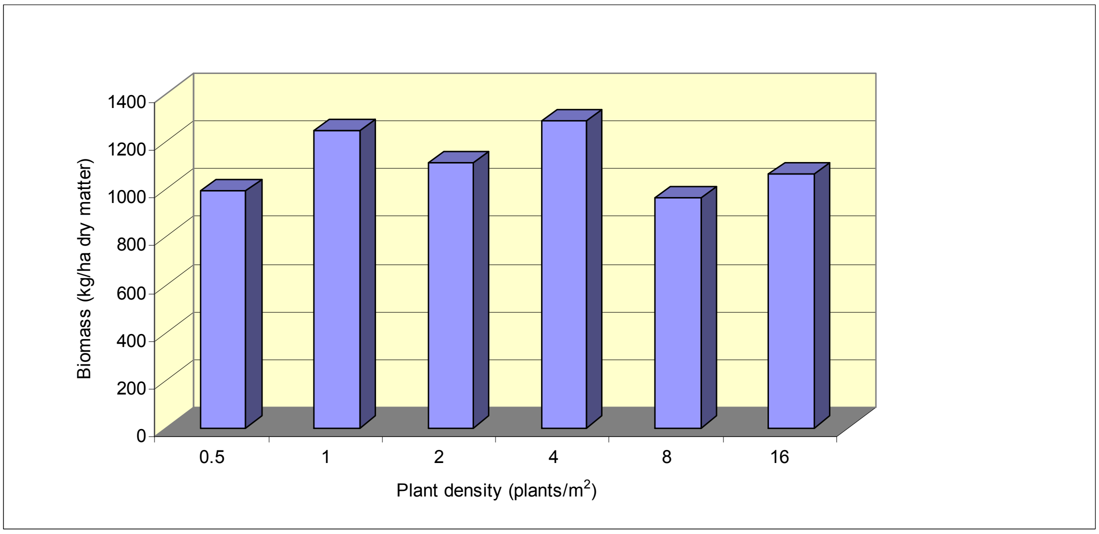


Figure 12. Cumulative panic grass biomass at Wellstead from October 2006 to June 2008 (seven cuts) when transplanted at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 (mean of 4 replicates)

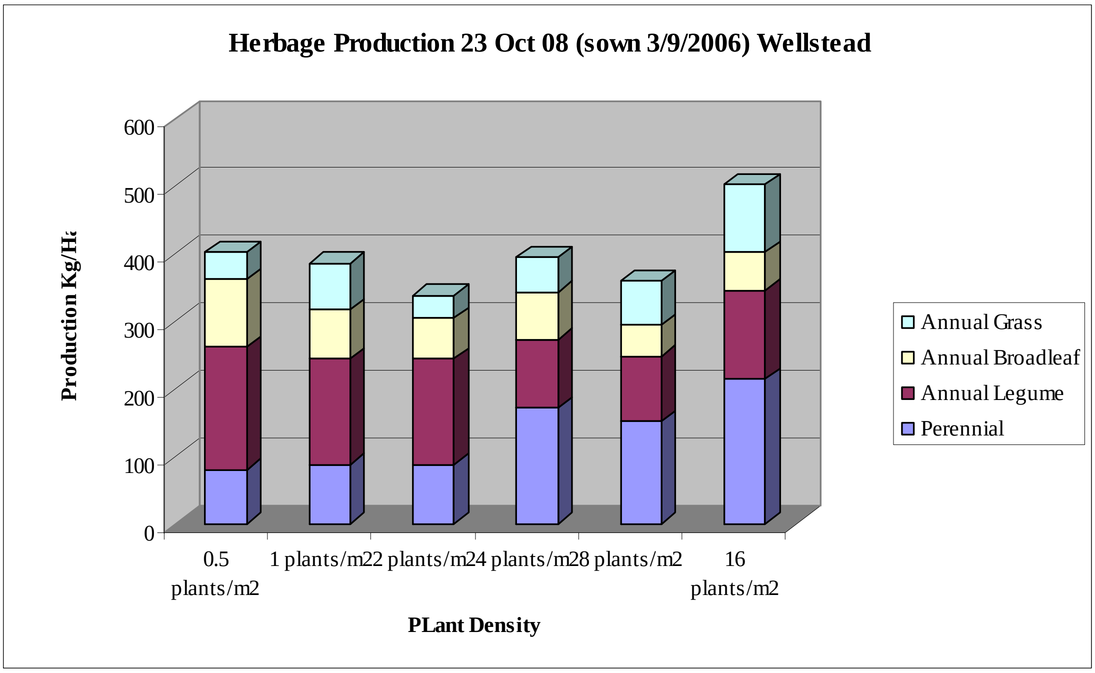


Figure 13. Biomass of panic grass, annual legumes, grasses and herbs from June 2008 to October 2009 after transplanting at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 at Wellstead (mean of 4 replicates). Edit graph!

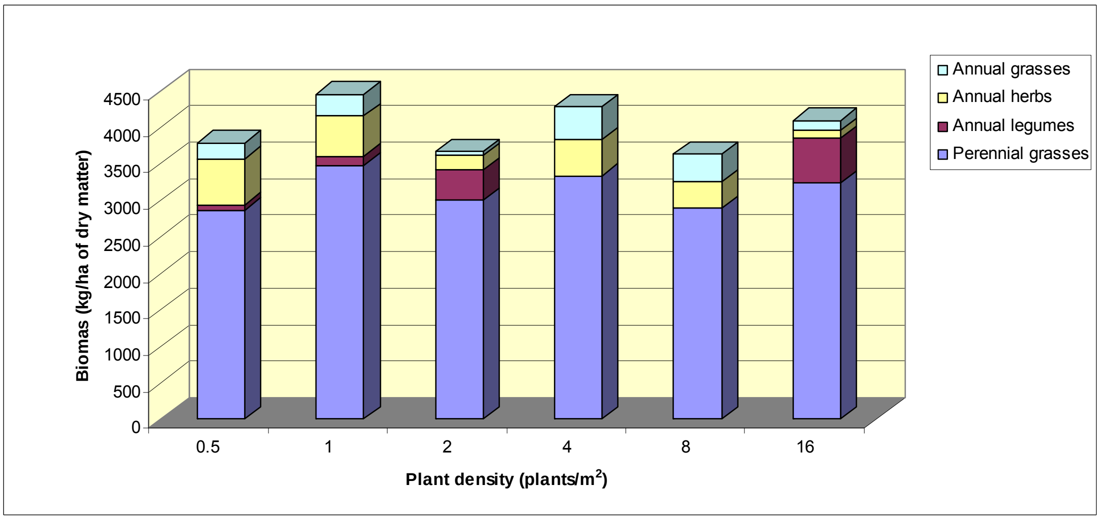


Figure 14. Cumulative biomass (total of six cuts) of panic grass, annual legumes, grasses and herbs from Feb 2009 to June 2010 after transplanting at densities of 0.5, 1, 2, 4, 8 or 16 plants/m2 at Wellstead. Results from only one replicate, due to destruction from inundation of the other three replicates in December 2008.

Analyses of feed quality on January 16, 2008 showed no clear relationships between feed quality parameters and density, apart from crude protein, which was higher in plants in the low density plots (Table 2).

Table 2. Feed quality parameters of panic grass on samples taken on January 16, 2008 in plots sown at densities of 0.5, 1, 2, 4, 8 or 16 plants/m^2 at Wellstead, WA. Analyses conducted by FEEDTEST Laboratories, Victoria.

Parameter	Panic grass density (plants/m^2)					
	0.5	1	2	4	8	16
Acid Detergent Fibre (%)	26	27.2	27	27.6	27.9	27.2
Ash (%)	12.3	13.3	11.8	13.5	14.6	12.2
Crude Protein (%)	17	15.1	14.4	13.2	12.1	12.9
Metabolisable Energy (MJ/kg)	8.8	8.8	9.3	8.2	8.3	8.8
In vitro digestibility (%)	60.5	60.3	63.4	57.3	57.8	60.7
Neutral Detergent Fibre (%)	60.2	60.2	59.2	60.4	60.2	60.4

Discussion

Panic grass plants grew much larger and were less stressed during late summer when planted at low densities and there was some evidence these plants had higher crude protein levels. Panic grass herbage production and total herbage production of the low density plots (0.5 and 1 plants/m^2) three years after sowing was lower than for the higher density plots, particularly at the Badgingarra site, where data was more reliable. This was also true for the highest density plots (16 plants/m^2), where it is apparent that strong competition between panic grass plants for moisture (and possibly other resources) limited sward productivity at this density.

On the basis of these results and more recent field observations, it appears that the optimum plant density three years after transplantation is in the order of 4-8 plants/m^2 for typical areas (400 – 600 mm mean annual rainfall) in the NAR and south coast suited to panic grasses. This is a compromise between high biomass production from the grasses in the warmer months and provision of sufficient space between plant crowns to allow germination and production from annual legumes and grasses in the winter months, when the panic grasses are largely dormant. This density could be increased in regions with more reliable summer rainfall or where there is access to summer irrigation. Conversely, the optimum density in low rainfall regions (<350 mm) may be closer to 1-2 plants/m^2.

These trials showed that once established the vast majority of panic grass plants will survive for at least three years and that survival is not affected by densities up to 16 plants/m^2. The main challenge is to get enough plants to survive through to the autumn after sowing and for this to occur evenly across the paddock.

4. Seed biology studies

4.1. Temperature requirements – determination of optimum sowing times

The interactions of temperature, light and moisture play a critical role in regulating seed germination in the field. A series of experiments was conducted to determine the optimum temperature for germination. This information provides a basis to estimate best seasonal "sowing windows" in the field to maximise germination and emergence. It also forms the basis for comparing the effects that physical and chemical germination enhancement treatments might have in modifying these "sowing windows."

A series of experiments was conducted to identify the optimum temperature for germination of a range of sub-tropical grasses and their influence on after-ripening dormancy. Germination under constant temperatures provides an indication of the effect of thermal accumulation on seed germination parameters, which can be used to gauge the effect of later sowing dates with higher average daily temperatures.

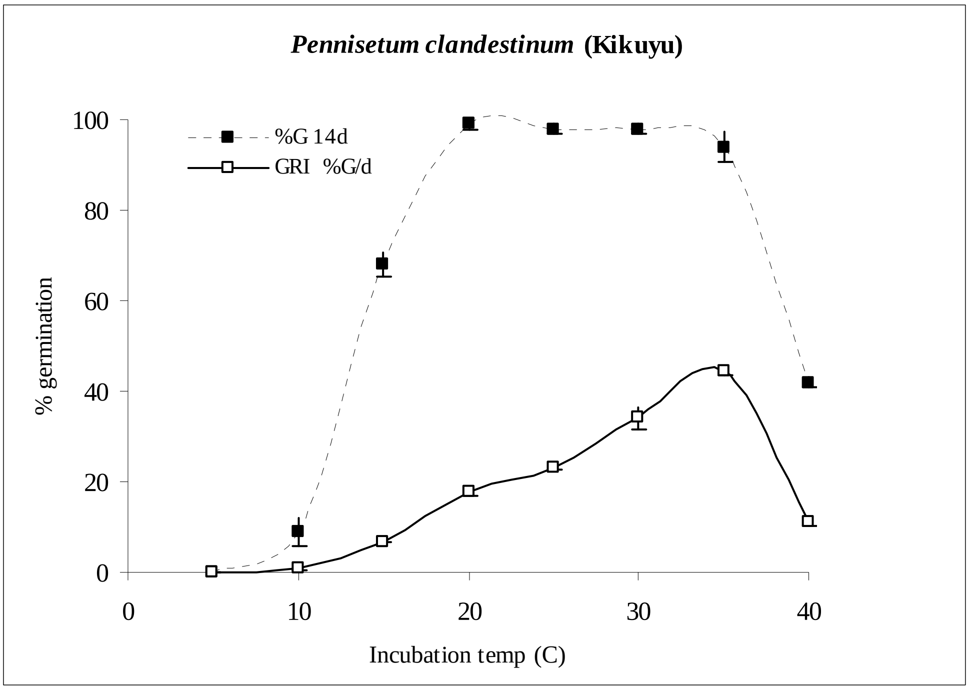
Materials and methods

Experimental seeds were stored for a minimum of 14 days before use under standard conditions of 16°C and 25% relative humidity. For comparative germination testing control seeds and primed seeds were stored and dried for 8-10 days beforehand under these standard conditions to ensure equivalent seed water contents. Seed was then cleaned with a vacuum aspirator. Filled seeds (those with well developed embryos) were identified by counting with a Faxitron MX-20 x-ray machine. This ensured that only 100% viable seed was used for experiments.

Seeds were incubated across a constant temperature gradient of 5-40 °C at 5 degree increments and at 18/5, 26/13 and 33/18°C alternating temperatures in a 12/12hr light/dark regime. These alternating temperature regimes were chosen to represent typical late winter, early to mid spring and late spring to early summer field temperatures in southern Australia. Four replicates of 25 seeds were plated into 90 mm Petri dishes with a water agar germination medium impregnated with 0.1% plant preservation mixture. Water agar was used to ensure seeds receive a constant water potential during germination. All dishes were sealed with plastic film to prevent evaporation and seeds were incubated in thermostatically controlled cabinets for a period of 14 days. Longer periods of incubation were avoided to negate seed aging effects on the sample. Light was provided by 30-36W florescent tubes emitting 8-20 µmol/s/m^2. Germination progress was scored every two days and seeds were considered to have germinated when root radicle protrusion exceeded 1 mm in length.

Results and discussion

Figure 15 shows a comparison of the effects of constant temperature on germination between the sub-tropical grass, kikuyu, with lucerne. Maximum germination for kikuyu occurred in the temperature range 20 - 35 °C, while maximum germination rate index, GRI, (% germinating seeds/day) was achieved at 35 °C. This contrasts with lucerne, which germinated at a maximum rate over a broad range of temperatures (10 -30°C). This means that kikuyu can only be sown in the warmer months, while lucerne can be sown be sown at the autumn break of season or from late winter onwards.



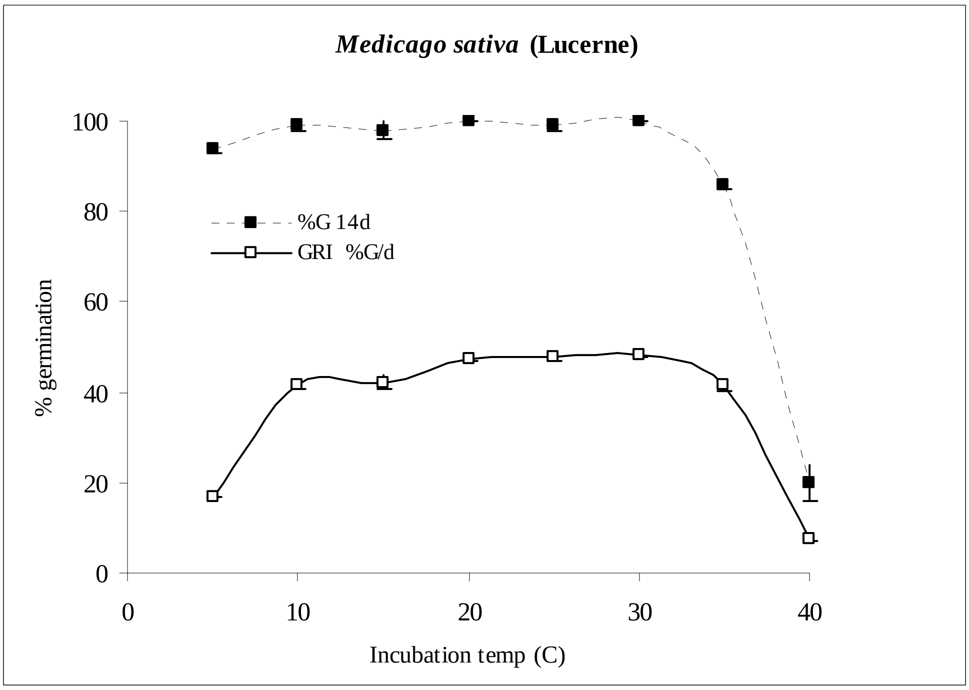


Figure 15. Germination curves showing maximal germination percent and germination rate over 14 days of kikuyu and lucerne over constant temperatures.

The optimum temperature regimes for germination of five sub-tropical grass species are presented in Table 3. Also shown are proposed “sowing windows” in the NAR of Western Australia for each species, based on comparing optimum temperature regimes with typical field temperatures. These “sowing windows” are a guide only and need to be modified for cooler and warmer regions of southern Australia. For example, optimum sowing dates will be 3-4 weeks later for the south coast of WA. Sowing times also need to be considered in the context of surviving summer drought and the ability of seedlings to compete with weeds and insects. So, while a species may be suited for germination in late winter to spring, root growth may not be sufficient to enable seedlings to survive summer drought. By and large, germination temperature regimes show that each of these species can be sown late winter to early spring, provided they can establish and survive the onset of the first summer.

In northern New South Wales and Queensland, the sowing window for sub-tropical grasses is much wider, which allows pasture establishment from early November to mid summer (Lodge and Harden 2009). Establishment can therefore occur under conditions of warm soils and the strong likelihood of summer rainfall.

Table 3. Constant temperature ranges and alternating temperature gradients for maximum germination percentage (%G) after 14 days for 5 sub-tropical grass species. The potential sowing window for the NAR of WA, corresponding to these temperatures is also shown (in yellow). Sowing dates will be later for cooler areas, souch as the south coast of WA.

Species	Optimum temperatures (^oC)		Potential sowing window[1]
	Constant	Alternating	
Megathyrsus maximum cv. Gatton	25-30	26/13-33/18	J F M A M J J **A S O N** D
Pennisetum clandestinum cv. Whitet	30-35	26/13-33/18	J F M A M J J **A S O N** D
Chloris gayana cv. Katambora	25-30	33/18	J F M A M J J **A S O N** D
Setaria splendida cv. Splenda	20-25	26/13-33/18	J F M A M J J **A S O** N D
Digitaria eriantha cv. Premier	20-25	26/13-33/18	J F M A M J J **A S O** N D

[1]Late sowing risky in southern Australia and dependent on plant water availability

4.2. Seed quality

Poor seed quality is often a major limitation to successful establishment of many target species. Sub-tropical grass seed lots can vary widely in quality in terms of germination, purity, dormancy and weed seeds. There are no national standards for seed quality, so it can be a case of ‘buyer beware’. Commercial seed batches often contain empty florets, immature seed, dormant seed and dead seed, in addition to viable seed. The germination of most sub-tropical perennial grass seed lots can vary from 10% to more than 60%, but is rarely higher than 70%. This compares with annual pasture legumes and grasses, where the germination is usually above 80%. An exception is kikuyu, which normally has germination >80%. Pure Live Seed (PLS) is a term used by some seed merchants as a measure of seed quality. PLS is calculated by multiplying the % purity by the % germination and then dividing by 10,000. Here, PLS = % purity x % germination/10,000, which gives a number between 0 and 1 (values closer to 1 indicate higher quality).

High quality seed is a batch containing not only high levels of seed purity, but also ready germinability. Three main factors affect seed quality: (i) seed fill at harvest time; (ii) the amount of inert harvest debris, empty and damaged seeds in the seed batch; and (iii) the duration and conditions of storage (Hopkins 1993). The amount of seed fill at harvest is affected by plant maternal environment, particularly the extent of fertilisation and abortion. Harvest timing is also critical and is a compromise between maximum seed set and the risk of seed shedding. Seed batches can contain empty florets, immature seed, dormant seed and dead seed in addition to viable seed; these amounts will form a higher proportion of the seed sample if the seed cleaning process is inefficient. Sufficient time is needed post-harvest to overcome any after-ripening seed dormancy issues. This is influenced by seed water content and ambient temperature, while extended lengths of storage under sub-optimal conditions can desiccate and decay seed embryonic tissue.

Seed quality attributes were determined in six seed batches used in the project to provide some information about quality issues.

Methodology

Seed quality was determined in seed batches acquired from commercial companies and from DAFWA seed increase plots. The commercial seed batches represented samples sold over the counter to farmers. The process for determining seed quality was as follows:

(i) Counting filled seeds (well developed embryos) in samples exposed to a Faxitron MX-20 x-ray machine (see Figure 16).

(ii) 14 day germination tests of cleaned seed samples.

(iii) Calculation of batch "seed purity" after seed cleaning.

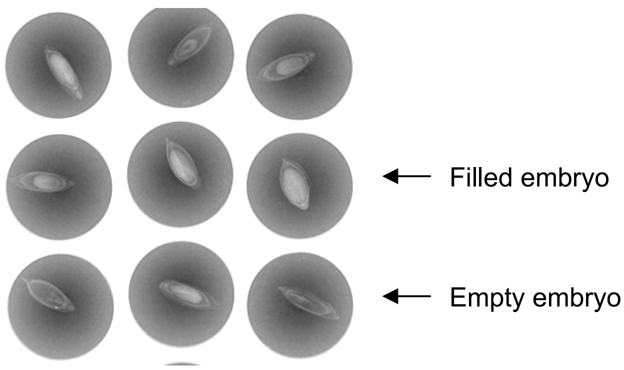


Figure 16. Plate from the Faxitron MX-20 x-ray machine showing filled (well developed embryos) and empty seeds of panic grass cv. Gatton

Results and discussion

Table 4 summarises the seed quality attributes of six commercial seed batches of sub-tropical grasses used in the project. Most of the sub-tropical grass seed batches had marginal to poor levels (< 65%) of seed purity. This was particularly the case with the Rhodes grass seed lot (38%). However, seed purity of the kikuyu seed batch was very high.

Table 4. Summary of seed quality attributes of commercial seed batches of sub-tropical grasses. Tests were undertaken after a minimum drying period of 14 days at 16°C and 25% relative humidity.

Species	Accession no.	% filled seeds[1]	% germination of filled seeds[2]	% seed purity by weight of viable seeds[3]
Panic grass cv. Gatton	020A-2006	42	65	60
Panic grass cv. Gatton	020B-2007	51	-	61
Kikuyu cv. Whittet	021B-2006	99	99	99
Rhodes grass cv. Katambora	022A-2006	18	88	38
Setaria cv. Splenda	023A-2006	37	13	52
Digit grass cv. Premier	024A-2007	36	86	59

[1]Percentage of fruits filled with seed as determined by x-ray analysis
[2]Percent germination after 14 days of fruits screened for >95% filled seed
[3]Seed purity of unscreened sample, denoted by percentage by weight of viable seeds

Preliminary observations suggest that morphological seed properties favouring separation from harvest debris and the threshing ability of seed cleaning equipment play an important role in the viability of cleaned seed. Kikuyu seed is relatively easy to separate from florets and other debris. Light seeds, seeds with awns and floret-encased seeds are difficult to separate without the use of expensive, finely-tuned cleaning equipment, such as vacuum aspirators. Unlike legumes, a visual assessment of seed purity is more difficult, as empty seeds or seeds encased in bracts or florets look the same as filled seeds. These factors contribute to the poor seed purity of many subtropical grass seed lots.

4.2.1. Optimum harvest times for sub-tropical grass seeds

The timing of seed harvest is critical for optimising seed quality. In common with many other perennial species that have asynchronous flowering, optimum harvest time of sub-tropical grass seed is a compromise between being late enough to maximise seed set (and minimise the proportion of immature seeds) and early enough to reduce the risks of shedding mature seeds. A range of plant and seed attributes were measured in order to develop a simple field assessment for determining the optimum time for harvesting sub-tropical grass seeds.

Irrigated grass plots of Gatton panic, Katambora Rhodes grass, Splenda setaria and Premier digit grasses at Medina Research Station, WA were visually scored at different stages of seed maturity for several characters, including the colour of seeds and flowering stems, seed firmness (using a “bite” test), extent of seed shedding and plot spikelet density. Seeds from scored plots were then assessed in the laboratory for seed fill (using an x-ray machine), seed hardness, colour and water content.

Gatton panic and Splenda setaria had poorer seed set than Katambora Rhodes and Premier digit grasses. Observations suggest seed set in the initial flowering flush of early summer was poor in each species, with high rates of abortion. Spikelets produced after cutting appeared to induce flowering with higher rates of seed set. The “bite” test, stem colour and seed water content were showed the most promise for determining seed ripeness and transferable high germinability in the seed.

4.3. Post-harvest seed dormancy

4.3.1. Effect of post-harvest storage conditions on germinability

Following harvest, many species, including panic grass, signal grass, digit grass and setaria (Hopkins 1993), display a period of post-harvest seed dormancy, sometimes referred to as *after-ripening* dormancy. In this state seeds are viable but are not able to germination. High levels of seed dormancy at sowing present a problem for two reasons. Firstly, establishment is likely to be much lower than expected given the seeding rate. Secondly, seeds that later come out of dormancy and germinate well after the optimum sowing window are less likely to successfully establish.

After-ripening dormancy is usually relieved over a period of time, whereby seeds undergo biochemical and physiological changes that enable them to eventually germinate. Seeds in this state are usually referred to as being in a state of ‘primary innate dormancy’. As seeds fulfil their after-ripening requirement, they then enter conditional dormancy, during which they tend to germinate over a narrow range of environmental conditions. During the progression of dormancy loss, the range of conditions over which the seed will germinate gradually widens, until it is able to germinate over the full range of conditions as dictated by the genotype.

The rate at which dormancy loss occurs has been linked to temperature and moisture conditions experienced by the seed. Non-dormant seeds of some species may re-enter dormancy if environmental conditions remain unfavourable for germination. Such seeds become conditionally dormant then eventually completely dormant. An understanding of these characteristics is important for the seed industry, particularly where storage and germinability of product are concerned.

Seed dormancy is a particular issue for sub-tropical grass seed coming into WA from northern Queensland, where most of the seed production in Australia is located. The time from harvest (January to April) to the recommended sowing time in WA of August – September may be insufficient for the seed to reach acceptable levels of germination. This differs from the situation in sub-tropical areas of eastern Australia, where sowing does not generally occur until November – February, by which time post-harvest seed dormancy has largely broken down.

An experiment was conducted to develop an understanding of the role of storage conditions on after-ripening dormancy in panic grass, compared to the non-dormant species, Rhodes grass.

Materials and methods

Freshly harvested seeds of Green panic and Katambora Rhodes grass were harvested from Medina Research Station on March 13, 2007. The seed lots were cleaned and graded to almost 100% viable seed, using the Faixtron X-ray machine. Seeds were then stored under three conditions:

(i) A laboratory at constant temperature of 18^{o}C;

(ii) A laboratory at room temperature; and

(iii) A garden shed (similar to a typical farm shed)

Seeds were sampled at regular intervals and germinated in Petri dishes for 14 days under alternating day/night temperatures of 26/13°C.

Results and discussion

Figure 17 shows germination results for the 12 months following seed harvest under the three different storage conditions. Germination of Green panic grass immediately after harvest was ~13%, indicting strong post-harvest dormancy. This was in contrast to almost 100% germination for Katambora Rhodes grass. Germination of Green panic increased over time under each storage condition, but required up to 7-12 months storage before high levels of germination. Green panic seeds stored under shed conditions exhibited the most rapid dormancy loss, with maximum germination being reached by November. This contrasted with seed stored at constant temperature, which did not reach maximum germination until January. Germination of both species stored in the shed declined somewhat after November, suggesting that high summer temperatures reduce viability. However, germination for Rhodes grass was still >80% after 12 months.

These results indicate that panic seed should not be used for sowing in late winter-early spring in the same year as the seed was harvested. For example, if this seed was sown in late August, <38% of seed would be expected to germinate, regardless of the storage treatment. Rhodes Grass, on the other hand, has no after-ripening requirement and seed can be sown in the year of harvest.

The most effective way of dealing with the issue of poor germination due to post-harvest seed dormancy in panic grass, setaria and signal grass is to store the seed until dormancy breaks down. Many wholesale seed merchants are unlikely to be willing to store seed of these species for more than 12 months after harvest, without substantially increasing seed price to cover additional storage costs. However, producers who want to sow seed of panic grass, digit grass, setaria or signal grass with maximum levels of germinability can purchase seed 12 months in advance of planned sowing and store them under dry conditions.

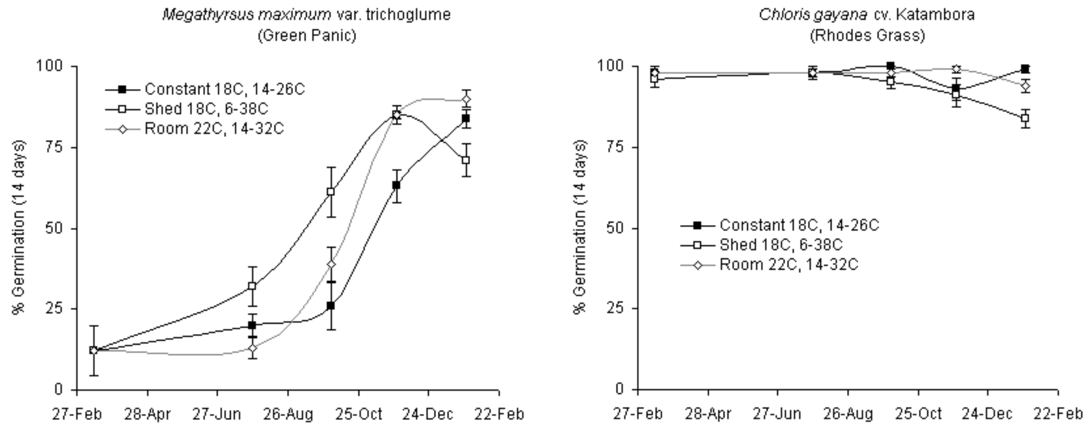


Figure 17. The effect of storage on germination for (a) Green panic (showing loss of post-harvest dormancy over time) and (b) Katambora Rhodes grass (which does not have post-harvest seed dormancy) from seed harvested on March 13, 2007. Seeds were either stored (i) in a laboratory at constant temperature of 18°C; (ii) a laboratory at room temperature; or (iii) a garden shed. Need to edit

One option for overcoming post-harvest seed dormancy is to prime seeds for 24 hours with plant signalling compounds. However, while these have been shown to work under laboratory conditions, their effects under field conditions are not as clear. The use of plant signalling compounds to enhance germination is discussed in Section 4.4.

4.3.2. Guidelines for purchasing panic grass seed

Figure 18 shows a decision support tree for farmers purchasing panic grass seed in south-western WA. With the development of agronomic practices for reliable establishment, the major limitation to success now appears to be poor seed quality. Firstly, there is the issue of post-harvest seed dormancy in the panic grasses. This arises because seed is commonly harvested in Queensland in February to April and farmers in WA plant in August-September, when post-harvest seed dormancy is still high. This is generally not an issue for panic grasses in sub-tropical areas of eastern Australia, where planting does not generally occur until November – February.

A more general seed quality issue is that of poor seed viability, with many commercial seed lots having less than 20% viable seed. In order for the seed industry to raise seed quality standards, a greater awareness is needed by the end-users of the seed (farmers and seeding contractors) of the quality of the seed they use for sowing. In this way, market forces are more likely to put pressure on the seed industry to increase standards. Consequently, an education program is needed to make growers aware of the issues. A simple 2-week bench-top germination test, using a pinch of seed placed on paper towelling in an ice cream or takeaway food container, has been designed to give growers some indication of the germinability of the seed they have purchased.

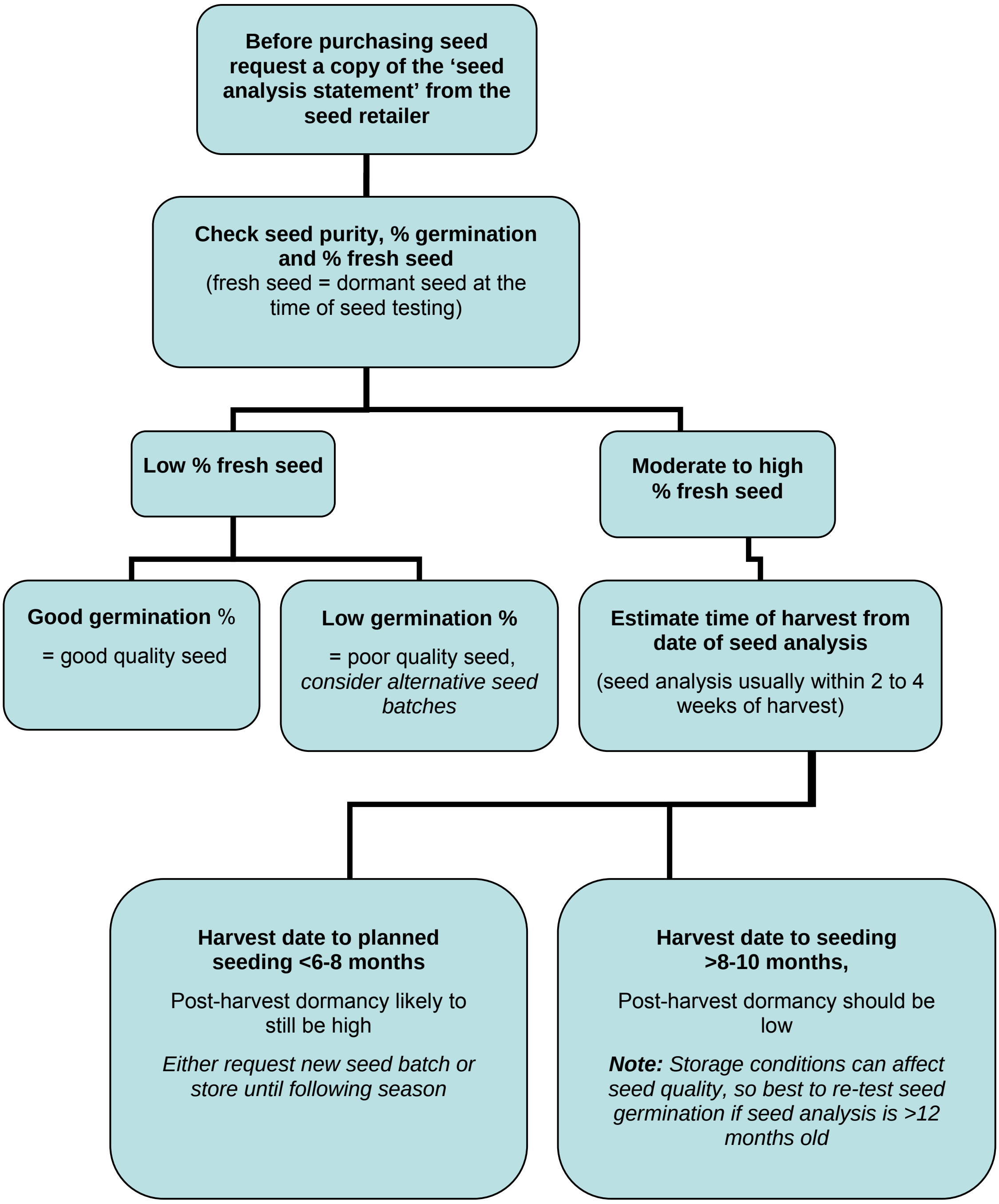


Figure 18. Decision support tree for farmers wishing to sow panic grass seed.

4.4. Use of plant signalling chemicals (chemical priming) to enhance germination and emergence

The germinability of a seed is regulated by its seed dormancy state and interactions with temperature, moisture and light. Priming (pre-germinating) seeds with water or germination stimulants has been successfully used for a range of species with plant signalling chemicals including karrikinolide (Flematti *et al.,* 2004), gibberellic acid (GA_3), benzoic acid (Senaratna *et al.*, 2003) and kinetin and salicylic acid (Senaratna *et al*., 2000). Kinetin is a cytokinin, a class of plant hormone that promotes cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement and auxiliary bud release and senescence. The function of this plant hormone requires auxins to be present. In contrast, salicylic acid is an inducer of systemic acquired resistance (SAR) to diseases in plants (Raskin 1992; Conrath *et al*. 1995) and is known to interact with plant respiration (Bourbouloux *et al*. 1998) and protein synthesis (Jin *et al*. 2000). Work by Dat *et al.* (1998) and Senaratna *et al.* (2003) shows that the use of salicylic acid increases thermo tolerance in seedlings. Unfortunately, the mode of action of these chemicals during germination remains unresolved. However, combined with other plant signalling chemicals, both kinetin and salicylic acid may provide additional support to improving germination, emergence and plant establishment.

The use of plant signalling compounds to promote germination and to overcome post-harvest seed dormancy in panic grass, digit grass and setaria was investigated in the laboratory and glasshouse and later under field conditions. The effects on promotion of germination were also examined in Rhodes grass and kikuyu, which do not have post-harvest dormancy. The advantage of laboratory-based experiments is that it allows a precise comparison of seed enhancing treatments under controlled conditions, which would be difficult to differentiate in field trials. While germination enhancement of 5-10% over controls may be statistically significant, such small gains may make little difference under field conditions. For this reason, enhancements or combinations of enhancements giving >20% over controls qualified as treatments for further consideration.

4.4.1. Plant signalling chemical agents – laboratory studies

Materials and methods

Seed lots were freed from debris using a vacuum aspirator and dried for a minimum of 14 days at 16°C and 25% relative humidity. For comparative germination testing control and primed seeds were stored and dried for 8-10 days beforehand under these conditions to ensure equivalent seed water contents. Seed lots were then checked for seed fill, using the Faixtron X-ray machine. Seeds for all experiments were >98% viable, confirmed using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

In order to test the efficacy of plant signalling chemicals, the critical moisture stress level for germination was calculated for each species. Critical moisture stress for seed was defined as the water potential where germination levels are 75% of maximum germination. The effect of moisture stress on germination was investigated by supplementing germination media with polyethylene glycol (PEG, molecular weight 8000), equating to the solute potentials of 0, −0.25, −0.5, −0.75, −1, -1.25, −1.5 and −2MPa. The osmotic potentials were calculated by equation 1, where x is the concentration of PEG_{8000} by %w/v (Michel and Kaufmann 1973).

$$\Psi_{PEG8000}\,(\mathrm{MPa}) = -7.6049x2 - 33.025x + 4.83 \qquad (1)$$

Four replicates of 25 seeds of five sub-tropical perennial grass species (panic grass cv. Gatton, kikuyu cv. Whittet, Rhodes grass cv. Katambora, setaria cv. Splenda and digit grass cv. Premier) were plated into 90 mm vented Petri dishes containing a filter paper with 8 mL of each solute potential. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20°C 12/12 hour light/dark constant incubator (TLMRIL model, Thermoline, QLD, Australia). This temperature was chosen as the osmolytic regression in equation (1) by Michel and Kaufmann (1973) and is correlated with temperature. Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index was calculated using equations 2 and 3. All germination data was arcsine transformed and an ANOVA was conducted to compare significance between treatments. Regressions and their equations in relation to the osmolytic gradient was plotted using CurveExpert 1.4. These equations were used to determine the critical moisture stress for each species at 20°C.

Final germination % at $t = [\Sigma G_t / r] \times 100$ (2)

Where: t = incubation period (days);

G = total germinated seeds across treatment petri dishes;

r = number of replicates

Germination rate index (GRI) = $\sum[(D_i - D_{i-1})/_i]$, (3)

Where: I = is the germination count day;

D_i = the percentage of seeds germinated at time "i";

D_{i-1} is the percentage of seeds adjudged germinated the previous count day (from Maguire, 1962).

Once the critical moisture stress levels had been determined, the effects of seven plant signalling agents (Table 5) on improving germination percentage and germination rate were investigated. Four replicates of 25 seeds were used in each treatment. Seeds were hydro-primed in chemical dilutions for 16-24 hours at 20 °C in the dark. Primed seeds and dry controls were dried on the laboratory bench for one day (at 22 °C and 55% relative humidity), then further dried for six days in a cool room set at 16 °C and 25% relative humidity.

Primed seeds were sown into 90 mm Petri dishes with a water agar germination medium impregnated and a 0.1% plant preservation mixture. Water agar was used to ensure seeds receive a constant water potential during germination. All dishes were sealed with plastic film to prevent evaporation and seeds were incubated for 14 days in thermostatically controlled cabinets at 18/5, 26/13 and 33/18 °C alternating temperatures under a 12/12h light/dark regime. Light was provided by 30-36W florescent tubes emitting 8-20 μmol.s^{-1}.m^{-2}. Germination progress was scored every two days and seeds were considered to have germinated when root radicle protrusion exceeded 1 mm in length.

Table 5. Germination enhancing chemicals used in experiments and their concentrations.

Seed enhancing agent	Concentration
Gibberellic acid (GA_3)	0.28 mM
Smoke water (SW)	1 %
Ethylene as ethaphon (Ethy)	20 mM
Karrikinolide (formerly Butenolide) (KAR_1)	0.67 µM
Potassium nitrate (KNO_3)	0.30 M
Kinetin (K)	0.05 mM
Salicylic acid (SA)	0.5 mM

Results and discussion

Table 6 describes the most effective germination enhancing chemicals. Significant germination enhancements (>20% relative to un-primed controls) were obtained for Splenda setaria with ethylene (Ethy). In particular, the chemical treatments appeared to break secondary dormancy in setaria seeds. Smaller germination enhancements were observed with karrikinolide (KAR1) for Gatton panic grass and a combination of Smoke water, and KAR_1 for Katambora Rhodes grass. Species with lower germination enhancements were those with germination rates >85% in control treatments, which did not require additional enhancement.

Table 6. Chemical germination enhancement effects in the laboratory on five sub-tropical perennial grass species. Treatments shown were all significantly different (*P*<0.05) to controls. %G denotes the maximum germination percentage after 14 days. GRI denotes the germination rate index (% germination per day).

Species	Un-primed seed		Best treatment[a]		Second-best treatment[a]	
	%G	GRI	%G	GRI	%G	GRI
			KAR_1			
Panic grass cv. Gatton	65	10	13	11		
			GA_3			
Kikuyu cv. Whittet	73	7	10	3		
			SW		*KAR_1*	
Rhodes grass cv. Katambora	85	10	12	35	12	29
			Ethy		*SW*	
Setaria cv. Splenda	13	2	55	12	50	10
			SW		*KNO_3*	
Digit grass cv. Premier	86	13	4	9	3	7

[a] KAR_1 = karrikinolide (0.67 μM), GA_3= gibberellic acid (0.28 mM), SW = smoke water (1%), ethy = ethylene (20 mM), KNO_3 = potassium nitrate (0.30 M)

4.4.2. Efficacy of 'best bet" plant signalling chemicals under field conditions

Micro-plot field trials were sown to examine if the individual "best bet" chemical signalling agents for each species found in the laboratory (Table 6) were transferable to the field. Emergence of seed treated with the "best bet" chemical enhancements in Table 6 were compared with un-primed and water-primed controls. These were conducted in parallel with laboratory germination studies sown at the same time.

Materials and methods

Two times of sowing were used for field trials. Field plots consisted of 50 seeds, while 25 seeds were used for laboratory controls. Treatments were replicated four times in each case. Germination of each species was compared in three treatments:

(i) Primed with the best chemical treatment from Table 6;

(ii) Primed in water for 24 hours; and

(iii) An un-primed control

Field plots were sown on August 28 and October 23 in 2007 at South Perth, WA. The design consisted of four replicates with main treatment effects in a Latin square design. The site was prepared using two knockdowns of glyphosate at 4 L/ha seven days prior to sowing. Plots were hand-sown in 50 cm rows. Sowing depth in the field was 7-9 mm. Irrigation was applied when necessary to prevent the soil drying out. Emergence was scored every 5 days for 30 days.

The August 28-sown trial contained Gatton panic grass, Katambora Rhodes grass, Splenda setaria, Premier digit grass and Whittet kikuyu, while the October 28-sown trial contained the first three species only.

All chemically treated seeds were primed for 24 hours. Primed seeds and dry controls were bench dried for three days (22^{o}C, 55% relative humidity) then further dried for six days in a cool room set at 16^{o}C, 25% relative humidity prior to sowing in the field. Laboratory controls were plated during the same week and placed into a 26/13 ^{o}C dark/dark cabinet.

Results

Tables 7 and 8 show emergence results for the August 28 and October 23 sowings, respectively. In the trial sown on August 23 significantly greater emergence than the controls occurred in the best treatments for Gatton panic grass and Katambora Rhodes grass (Table 7). Water priming doubled emergence of panic grass, while both water and smoke water primed treatments doubled emergence and emergence rate of Rhodes grass. There was a significant increase in emergence of setaria seeds primed with ethylene, compared to un-primed controls, but increases in emergence were not found in the field with the best bet chemical treatments for the other species. This was not unexpected, given that field temperatures were below the optimum germination range for these species, a factor previously shown to be important for differentiating chemical priming effects.

Table 7. Field emergence and laboratory germination over 30 days following sowing on August 28 of five sub-tropical perennial grasses. ERI = emergence rate index (% emerged seeds per day) and GRI = germination rate index (% germinated seeds per day). * denotes significant differences (*P*<0.05) between primed and un-primed treatments within species. Values in parenthesis represent +/- one standard error.

Species	Priming treatment[a]	30 day field emergence		30 day lab germination	
		% emerged (E)	ERI (%E/day)	% germinated (G)	GRI (%G/day)
Panic grass	Un-primed	21.0 (6.3)	1.2 (0.4)	66.0 (5.7)	7.9 (1.1)
	H_2O	39.0 (6.1)*	2.4 (0.4)*	89.0 (3.0)*	11.5 (0.6)*
	KAR_1	32.5 (5.9)	2.0 (0.3)	97.0 (1.0)*	13.1 (0.7)*
Kikuyu	Un-primed	38.5 (10.5)	2.3 (0.7)	97.0 (1.9)	18.6 (0.5)
	H_2O	40.5 (9.9)	2.6 (0.7)	99.0 (1.0)	19.6 (0.2)
	GA_3	47.0 (11.4)	3.1 (0.7)	100.0 (0.0)	19.7 (0.2)
Rhodes grass	Un-primed	11.5 (1.9)	0.9 (0.2)	94.0 (1.1)	18.2 (0.4)
	H_2O	22.5 (8.5)*	1.8 (0.7)	97.0 (1.0)*	18.3 (0.4)
	SW	25 (5.4)*	2.2 (0.5)*	97.0 (1.9)	19.3 (0.3)
Setaria	Un-primed	14.5 (3.2)	0.8 (0.1)	26.0 (6.0)	2.0 (0.3)
	H_2O	19.0 (5.2)	1.3 (0.4)	79.0 (5.7)*	8.3 (0.5)*
	Ethy	21.5 (3.6)*	1.3 (0.3)*	90.0 (3.5)*	10.3 (0.6)*
Digit grass	Un-primed	25.5 (4.3)	1.6 (0.3)	89.0 (4.4)	14.6 (0.7)
	H_2O	34.0 (7.8)	2.4 (0.5)	94.0 (2.0)	14.7 (0.6)
	SW	30.0 (6.2)	2.0 (0.5)	93.0 (1.9)	16.7 (0.2)*

[a] KAR_1 = karrikinolide (0.67 μM), GA_3= gibberellic acid (0.28 mM), SW = smoke water (1%), ethy = ethylene (20 mM)

In the October-sown trial, laboratory priming results were transferable to the field in each species, but at a significantly reduced level compared with laboratory conditions (Table 8).

Table 8. Field emergence and laboratory germination over 30 days following sowing on October 23 of Splenda setaria, Katambora Rhodes grass and Gatton panic grass. ERI = emergence rate index (% emerged seeds per day) and GRI = germination rate index (% germinated seeds per day). * denotes significant differences (*P*<0.05) between primed and un-primed treatments within species. Values in parenthesis represent +/- one standard error.

Species	Priming treatment[a]	30 day field emergence		30 day lab germination	
		% emerged (E)	ERI (%E/day)	% germinated (G)	GRI (%G/day)
Panic grass	Un-primed	21.0 (6.3)	1.2 (0.4)	79.0 (4.4)	7.9 (0.5)
	H_2O	39.0 (6.1)*	2.4 (0.4)	85.0 (3.4)	11.5 (0.7)
	KAR_1	32.5 (5.9)*	2.0 (0.3)	90.0 (2.6)*	12.5 (0.7)
Rhodes grass	Un-primed	54.0 (7.1)	3.3 (0.5)	97.0 (1.9)	15.4 (0.2)
	H_2O	68.0 (6.3)	6.4 (0.5)	86.0 (2.6)*	12.8 (0.6)
	SW	76.5 (9.2)*	9.1 (1.5)	95.0 (2.5)	14.5 (0.5)
Setaria	Un-primed	31.0 (3.1)	1.8 (0.2)	18.0 (3.8)	1.3 (0.4)
	H_2O	24.5 (3.5)	1.4 (0.2)	67.0 (5.5)*	8.4 (0.9)
	Ethy	44.0 (7.7)*	2.9 (0.4)	84.0 (4.3)*	12.7 (0.9)

[a] KAR_1 = karrikinolide (0.67 μM), SW = smoke water (1%), ethy = ethylene (20 mM)

4.4.3. Plant signalling chemical combinations (PSCCs)

Combinations of plant signalling agents were examined, to determine whether their different modes of action could have complementary effects on germination. In particular, the additive effects of kinetin and salicylic acid on best-bet treatments were examined. Kinetin is a cytokinin, a class of plant hormone that promotes cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement and auxiliary bud release and senescence. The function of this plant hormone requires auxins to be present. In contrast, salicylic acid is an inducer of systemic acquired resistance (SAR) to diseases in plants (Raskin 1992; Conrath *et al*. 1995) and is known to interact with plant respiration (Bourbouloux *et al*. 1998) and protein synthesis (Jin *et al*. 2000). Work by Dat *et al.* (1998) and Senaratna *et al.* (2003) shows that the use of salicylic acid increases thermo tolerance in seedlings. Unfortunately, the mode of action of these chemicals during germination remains unresolved. However, combined with other plant signalling chemicals, both kinetin and salicylic acid may provide additional support to improving germination, emergence and plant establishment.

Materials and methods

Seeds were primed for 18 hours in treatments, consisting of combinations of chemical signalling agents at the concentrations shown in Table 6, after which they were rinsed under water and patted dry with a paper towel. Primed seeds were then left to bench dry for two days at 22°C, 55% relative humidity, then transferred to a 16°C, 25% relative humidity room to further dry for another four days. Four replicates of 25 seeds of each treatment were plated into 90 mm vented Petri dishes containing a filter paper to which 8 mL of the critical moisture stress solute potential for each species was added. Controls consisted of treated seed plated into Petri dishes with filter paper containing only water. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20^{o}C dark constant incubator (TLMRIL model, Thermoline, QLD, Australia).

Germination was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index across treatments were calculated using equation 2 and 3. All germination data was arcsine transformed and an ANOVA was conducted to compare significance between treatments. This enabled identification of optimum plant signalling chemical combinations (PSCC) for glasshouse and field trials.

Results

Two plant signalling chemical combinations that significantly increased germination, using moisture stress to differentiate treatments, were Ethy+SA for panic grass and KAR+SA+K for setaria (Table 9). The chemical combinations of Ethy+SA+K for kikuyu, SW+SA+K for Rhodes grass and SW+ SA for digit grass also had higher germination than controls but these differences were not significant.

Table 9. Optimum plant signalling chemical combinations for improved germination of five sub-tropical perennial grass species, using moisture stress to differentiate control (no treatment) and priming treatments. Degree of significance between control and priming treatments are denoted by asterisks where * = *P* <0.05, **= *P* <0.01 and * = *P* <0.001. Standard errors are in parenthesis.**

Species	Best treatment[a]	% germination after14 days at CMS[b]		
		Control	Best treatment	*P*
Panic grass	Ethy+SA	3 (0.4)	16 (3.3)	**
Kiuyu	Ethy+SA+K	16 (3.6)	19 (4.9)	ns
Rhodes grass	SW+SA+K	23 (4.4)	28 (3.9)	ns
Setaria	KAR_1+SA+K	7 (1.0)	16 (2.2)	**
Digit grass	SW+SA	11 (0.7)	15 (1.1)	ns

[a]Ethyl = ethylene (20 mM), SA = salicylic acid (0.5 mM), K = kinetin (0.05 mM), SW = smoke water (1%), KAR_1 = karrikinolide (0.67 μM),

[b]Critical moisture stress, defined as the water potential that retards germination by 75% of maximum germination

4.4.4. Efficacy of plant signalling chemical combination effects under glasshouse and field conditions

Materials and methods

Three sets of treated seeds of Katambora Rhodes grass, Gatton panic grass, Splenda setaria, Whittet kikuyu and Premier digit grass were prepared, one each for glasshouse and field trials and one for laboratory controls.

The following treatments were prepared: priming with H_2O, priming with the optimum chemical signalling chemical combination for each species (shown in Table 9) and no treatment (control).

Field trials were sown on September 18, 2008 into a free-draining sandy soil at the University of Western Australia Shenton Park Field Station. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha (a.i. 680 g/L), three and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeds were hand-sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a Latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored for emergence weekly for 30 days, then fortnightly over a 120 day period.

The laboratory controls were run on the 10 September 2008 using the laboratory germination methodology described in previous sections. An alternating 26/13^{o}C 12/12 hour temperature regime was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark.

Glasshouse trials were sown on 21 May 2009 at 5-7 mm depth into 150 mm diameter pots containing free draining white sand to a 140 mm depth. Four replicates of 50 seeds of each treatment were used. An alternating 27/15^{o}C 12/12 hour temperature regime was used. Pots were irrigated daily and emergence was scored weekly over a 120 day period.

Results

Under laboratory conditions, plant signaling chemical combinations improved germination significantly to controls in panic grass, Rhodes grass, setaria and digit grass (Table 10). Under glasshouse conditions, chemical combinations improved emergence significantly after 30 days in panic grass, kikuyu, digit grass and setaria. However, there was a general lack of transfer of the successful laboratory and glasshouse treatments to the field environment. Under field conditions, only panic and digit grasses showed significant improvements compared with controls in 30-day emergence results (Table 3). Under laboratory conditions, Gatton panic grass displayed very strong physiological dormancy. When it was treated with the plant signaling chemical combination of ethylene +salicylic acid, the priming benefits were carried through to field emergence.

Table 10. Percent germination in the laboratory after 14 days and percent emergence in the glasshouse and field after 30 days of sub-tropical perennial grass seeds primed with water (H_2O), primed with the optimum plant signalling chemical combination (PSCC) shown in Table 7, compared with untreated controls. Standard error is shown in parenthesis. Within each environment, PSCC treatments within a species significantly different (*P* <0.05) from controls are denoted by an asterisk (*)

Species	Laboratory			Glasshouse			Field		
	Control	H_2O	PSCC	Control	H_2O	PSCC	Control	H_2O	PSCC
Panic grass	9 (1.0)	45 (3.8)	83 (2.3)*	11 (1.3)	34 (2.9)	56 (2.2)*	11(1.3)	34 (2.9)	56 (2.2)*
Kikuyu	89 (1.7)	89 (2.3)	95 (2.6)	23 (5.6)	48 (2.7)	45 (3.8)*	38 (7.2)	36 (1.0)	40 (4.2)
Rhodes grass	20 (3.0)	28 (7.5)	73 (8.6)	35 (6.5)	51 (1.7)	46 (10.1)	64 (4.5)	51 (10.7)	67 (9.8)
Setaria	53 (4.7)	41 (4.3)	79 (4.6)*	28 (2.9)	33 (5.6)	40 (3.4)*	26 (4.3)	36 (7.9)	36 (8.2)
Digit grass	79 (1.3)	76 (5.4)	100	30 (4.6)	50 (6.3)	71	31 (4.1)	52 (4.6)	47

	(0.0)*	(6.5)*	(7.3)*

Discussion

These experiments showed that the use of plant signaling chemical combinations can improve the germination and emergence of sub-tropical perennial grasses under laboratory conditions and this is often transferred to the glasshouse. However, there was a general lack of translation of this benefit to field emergence, except in Gatton panic grass, which had significant physiological dormancy at the time of treatment. These results suggest that whilst the use of plant signaling chemicals are useful in improving germination percentage, rate and germination tolerance to moisture stress, there needs to be a greater understanding of the biological and environmental factors that reduce this benefit under field conditions.

The efficacy of plant signaling chemicals in glasshouse and field conditions could be dictated by several factors: chemical dependencies, dormancy status of seed and stability of chemicals to temperature.

The use of kinetin may be an example of a chemical dependency. Here the efficacy of salicylic acid and kinetin to improve germination under moisture stress in laboratory conditions was a common theme across species. However, the use of kinetin in combination did not always work. Kinetin, a cytokinin, induces cell division but requires auxin to be present in order for it to be effective and the ratio of auxin to cytokinin is crucial during cell division (Mok and Mok, 1994). Auxins in plant tissues promote the production of ethylene, which along with GA3, interact with how seeds utilise light as a cue for germination. The efficacy of kinetin in species that did not respond significantly to the chemical may be due to a lack of auxin in the seed of those species.

The efficacy of chemicals on germination and emergence benefits can be affected by the dormancy status of seeds at the time of treatment. For example, the efficacy of GA_3 as a plant signaling chemical appears dependant on after-ripening status (Hilhorst and Karssen, 1992). Gibberellic acids are not directly responsible for breaking down dormancy, but in combination with after-ripening relief, their inclusion aids the development of a GA-responsive system. GA biosynthesis in seeds occurs during seed imbibition and functions independently from dormancy (Karssen and Lacka, 1986).

Only panic grass responded well to PSCCs under field conditions. Low germination in the laboratory of untreated seed showed it to be highly dormant (most probably related to primary dormancy), but was highly responsive to ethylene. For panic grass, it could be that ethylene acts independently of after-ripening status. This conclusion is based on observations that ethylene breaks dormancy of two month-old seed much better than GA_3, yet in seven month old seed, the reverse occurs (C. Loo, unpublished data). These observations need further investigation.

The stability of novel plant signaling chemicals, such as GA_3, kinetin, salicylic acid, smoke water, ethylene and karrikinolide, under field conditions has not been studied. Most of these chemicals appear remarkably stable, with >100^{o}C temperatures required for chemical decomposition (O'Neil, 2001), although there is no knowledge on karrikinolide and smoke water in this respect. Ethylene, on the other hand, is exceptionally volatile (O'Neil 2001). For ethylene to function as a dormancy breaker on panic grass under field conditions, it appears that incorporation of the chemical into the seed through hydro-priming will work much better than an external coating on the seed. Improved emergence of digit grass with use of smoke water shows that this compound is stable and effective in the field. Similar field benefits in emergence from the use of smoke water have been observed in *Stylidium affine* (S. Turner, unpublished data).

The potential to observe chemical hydro-priming benefits on seed germination and emergence is dependent on sampling frequency, experimental duration and optimal temperatures for germination and emergence. For many non-dormant species, the priming benefit is largely due to increases in the rate of germination and emergence. Hence, benefits from these chemicals occur within the first few days after imbibition and differences between priming and control treatments diminish with time.

These experiments suggest that potential field application of chemical hydro-priming appears limited to species which display highly dormant seed at the time of treatment, notably in panic grass and digit grass. While this appears to benefit emergence in such species, carryover to plant establishment and first year survival remains unresolved. The use of this enabling technology has potential to increase field emergence, but the lack of translation to the field environment suggests that there are other variables relating to priming that need to be understood. Future research should focus on developing an understanding of seed and chemical integrity after priming and how this relates to the seedbed environment.

5. Agronomy studies

5.1. Sowing time

In sub-tropical areas of northern New South Wales and Queensland (and other areas of the sub-tropics), sub-tropical perennial grasses are typically sown from late spring to early summer (Lodge and Harden 2009). Establishment, therefore, occurs when there is a strong likelihood of summer rainfall and when soil temperatures are optimum for germination (Table 3). However, in Mediterranean climates, such as south-western Australia, a summer drought period of 4-8 months occurs, with highly sporadic and very unreliable rainfall. Sowing time in these environments is, therefore, a compromise between sowing early enough to enable sufficient root development prior to onset of the summer drought and late enough so that low soil temperatures do not limit germination and growth (see Table 3).

In order to test the principles of the sowing window generated by the laboratory studies in Table 3, micro-plot field trial was established at Gillingarra, WA (~150 km north of Perth). The existing guidelines for the Gillingarra district were to sow in early spring, when the average soil temperature was above 15°C, but there was no data to support this. The trial examined the effects of annual weed competition and plant survival, and measured plant growth dimensions and dry matter production.

Materials and methods

The trial was conducted in 2007 on a sandy duplex soil at Gillingarra, 35 km south of Moora, WA (30°57’00”S, 115°59’25”E), to determine the optimum sowing time to maximise establishment of Gatton panic and Katambora Rhodes grass. The perennial legume *Lotononis bainesii* cv. Miles was also included for comparison.

Ten weekly sowing dates, from July 25 to September 26, were used. Plots consisted of five 2 m rows, spaced 0.5 m apart. These were hand-sown with 100 evenly placed seeds (identified as containing embryos using the Kings Park Faxitron MX-20 x-ray machine), sown to a depth of 5 mm. The central three rows of each plot were sown to the three species, with the outer rows consisting of Gatton panic grass grown as a buffer. Treatments were replicated four times and the trial was arranged in a randomised complete block design.

The trial area was sprayed with glyphosate @ 2L/ha on July 15 and again one week prior to each weekly sowing. This routine occurred to the 6th sowing (August 29), after which all remaining unsown plots were sprayed.

Emerged seedlings were counted weekly until October 24 and then monthly until January 31, 2008. Weed densities were visually rated on October 24 using a 1-5 scale, where 1 = low, 3 = moderate and 5 = very high. Plant width was measured on five randomly chosen seedlings per plot on October 24. Soil temperatures were measured with temperature probes placed 5 mm under the soil surface.

Figure 19 shows the Gillingarra trial site at a field day held on November 2007.

Figure 19. Field day at Gillingarra in November 2007 showing the establishment resulting from different sowing times of sub-tropical perennial grasses

Results and discussion

Monthly rainfall totals and mean maximum and minimum temperatures from July 1, 2007 to March 31, 2008 at Moora (20 km north of the Gillingarra site) are shown in Figure 20. Soil temperatures are shown in Table 11. The site received approximately 130 mm rainfall from July to September, which was below the average of 201 mm. This was sufficient for germination at each sowing date, except for September 26, which was sown into dry soil.

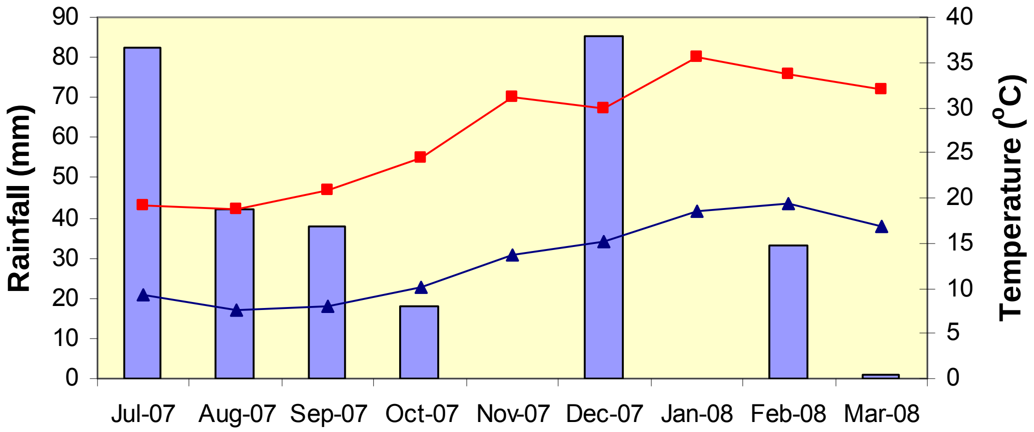


Figure 20. Monthly rainfall totals (bars) and mean maximum (red) and minimum (blue) temperatures from July 1, 2007 to March 31, 2008 at Moora, WA, 20 km north of the Gillingarra site (Data from the Australian Bureau of Meteorology).

Maximum soil temperature rose above 15°C in the week following the first time of sowing on July 25 (Table 11). However, more than 10 hours per day of temperatures above 15°C did not occur until the week of August 22.

Table 11. Mean soil (5 mm below the surface) maximum and minimum temperatures (°C) and number of hours per day > 15 °C in the seven days following sowing for 10 weekly sowing times at Gillingarra, WA.

Sowing date	Minimum temperature (°C)	Maximum temperature (°C)	Hours/day >15°C
July 25	8.5	17.0	6.0
August 1	10.0	18.5	6.5
August 8	10.5	17.5	7.5
August 15	9.0	18.5	8.0
August 22	9.0	22.5	10.0
August 29	7.0	24.0	10.5
September 5	9.5	25.0	10.5
September 12	8.5	23.5	11.5
September 19	10.0	25.5	10.5
September 26	13.0	27.0	13.0

Winter weeds, such as capeweed and annual ryegrass, continued to germinate in high densities during July and August, so that grasses emerging from the first two sowings had to compete with a high annual weed density (Table 12, Figure 21). The best weed control was achieved when the second knockdown was applied on or after August 8 (Table 12).

Table 12. Visual weed density ratings (1-5), measured on October 24, in plots sown to Gatton panic, Katambora Rhodes grass and *Lotononis bainesii* at 10 weekly intervals at Gillingarra, WA. All plots were initially sprayed with glyphosate @ 2L/ha on July 15, with a second application one week prior to each sowing date until the 6th sowing (August 29), after which all remaining unsown plots were sprayed.

Sowing date	Second spray date	Weed density (1-5 rating)*
July 25	July 18	5
August 1	July 25	3
August 8	August 1	2
August 15	August 8	1
August 22	August 15	1
August 29	August 22	1
September 5	August 22	1
September 12	August 22	1
September 19	August 22	1
September 26	August 22	1

*1-5 visual rating, where 5 = very high weed density, 3 = moderate density and 1 = low density.

Figure 21. Plates showing establishment of rows of Rhodes grass (R), panic grass (P) and *Lotononis bainsii* (L) and annual weed residues from sowing on July 25, August 15 and September 12 in 2007 at Gillingarra, WA. Photo taken on November 1, 2007.

Large differences in establishment for each species were observed between sowing times. Although seeds germinated from the earliest sowing date (July 25), newly emerging seedlings were visually stressed and only 80% survived (Table 13). A key factor for establishment related to the number of hours when soil temperatures were above 15°C. Maximum emergence occurred after the week of August 22, when soil temperature was above 15°C for more than 10 hours per day.

New panic grass seedlings emerged between 21 and 42 days after sowing (DAS) for the first four sowings (July 25 – August 22), indicating a delay in germination from these sowing dates, presumably due to seed dormancy effects (Table 13). For the July 25 sowing, twice as many panic grass seedlings had emerged 42 DAS, compared to 21 DAS. This delay in germination was also seen for Lotononis, but not for Rhodes grass (Table 13).

Maximum panic grass plant numbers established from August 29 (Table 13, Figure 22). Rhodes grass and Lotononis seedlings also had high survival rates throughout the August sowings, with both having highest plant numbers when sown on August 15 (Table 13, Figure 22). The percentage of seedlings emerged at 21 DAS that were still present at 42 DAS declined after the August 22 sowing date (Table 13). Seedling emergence was much lower from mid-late September and a much higher proportion of emerged seedlings failed to survive for 42 DAS for each species. The soil surface on September 26 was dry, resulting in very poor establishment and seedling survival of each species (Table 13, Figures 22 and 23)

On October 24 plant size was highest for both panic grass (Figure 22) and Rhodes grass (Figure 23) sown on August 22. Plant size declined steadily with each sowing date. Plants of panic grass on September 26 were only 25% the size of plants sown on August 22, while Rhodes grass plants were only 11% the size.

Table 13. Seedling counts (emerged seedlings from 50 germinable seeds) at 21 and 42 days after sowing (DAS) and percentage survival (seedlings present at 42 DAS that had emerged at 21 DAS) for Gatton panic grass, Katambora Rhodes grass and *Lotononis bainsii* cv. Miles from ten weekly sowing times in 2007 at Gillingarra, WA.

Sowing date	Gatton panic grass			Katambora Rhodes grass			*Lotononis bainsii*		
	21 DAS	42 DAS	% survival	21 DAS	42 DAS	% survival	21 DAS	42 DAS	% survival
July 25	4.0	8.1	100[1]	19.6	9.8	50	5.9	5.6	95
August 1	4.6	11.7	100[1]	11.0	10.8	98	3.3	8.8	100[1]
August 8	5.2	8.8	100[1]	19.4	16.9	87	5.5	7.1	100[1]
August 15	9.9	10.5	100[1]	23.0	20.0	87	5.9	8.5	100[1]
August 22	10.1	13.2	100[1]	19.3	17.6	91	2.0	8.0	100[1]
August 29	19.7	16.3	83	15.3	13.6	89	4.3	3.4	79
September 5	13.5	9.3	65	20.6	12.4	60	9.1	7.9	87
September 12	14.3	8.3	58	21.3	10.0	47	7.6	5.8	76
September 19	12.8	7.1	55	19.8	10.4	53	8.4	5.9	70
September 26[2]	1.1	0.3	27	19.0	7.4	39	0.8	0.1	13

[1]Additional seedling emergence between 21 and 42 DAS

[2]Sown in to dry soil

Plant counts of panic grass on January 31, 2008 (Figure 24) mirrored the same trends as the emergence counts taken at 42 days after each sowing (Figure 22). However, plant counts on January 31, 2008 for Rhodes grass (Figure 25) showed very little difference between the times of sowing, apart from the much lower density of the earliest sowing. However, unseasonable rains of more than 80 mm in December (Figure 20) would have had the effect of increasing survival of the small seedlings from the later sowings. It is likely that far fewer seedlings sown on September 19 and 26 would survive a more typical summer at Gillingarra (see Figure 35 for the 2009-10 summer period).

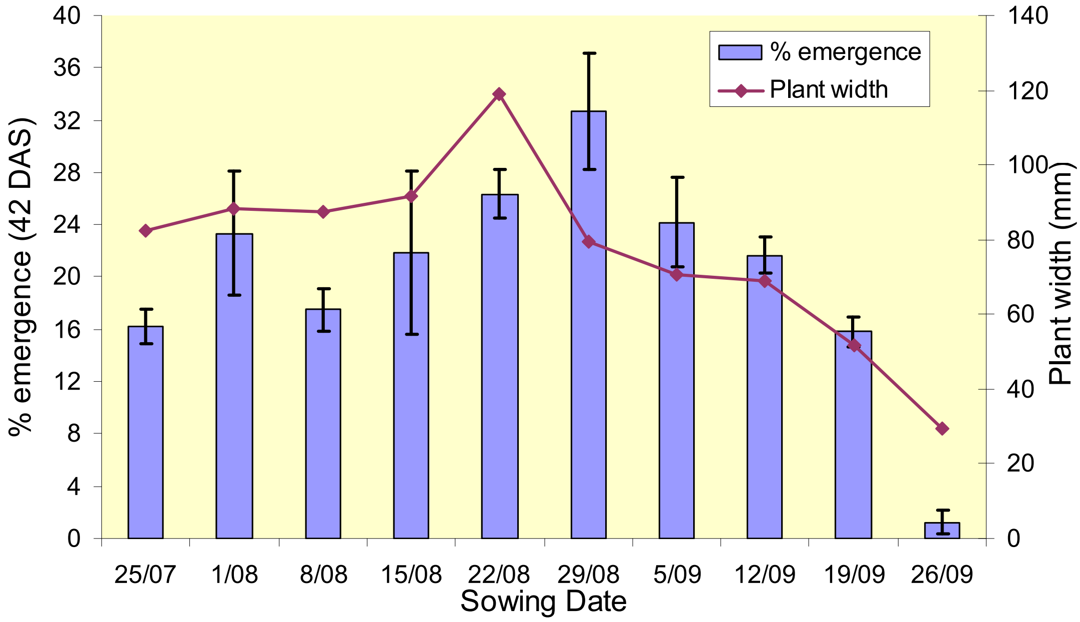


Figure 22. Panic grass seedling emergence (percent of germinable seeds sown) 42 days after sowing (DAS) and plant width (mm) on October 24 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors for % emergence.

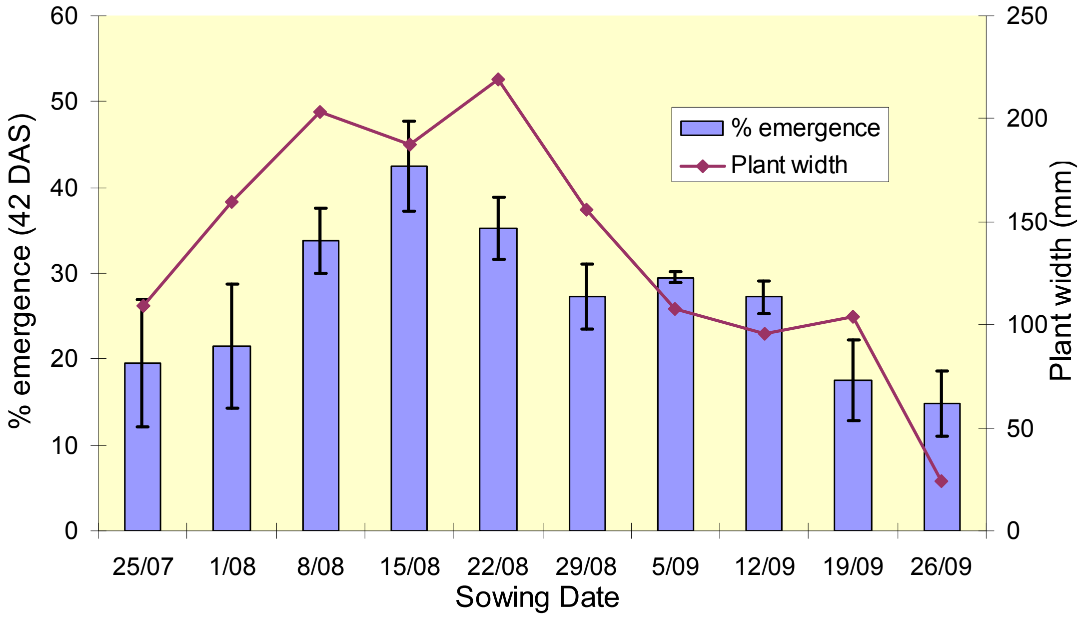


Figure 23. Rhodes grass seedling emergence (percent of germinable seeds sown) 42 days after sowing (DAS) and plant width (mm) on October 24 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors for % emergence.

These results show the importance of sowing at the right time for optimising sub-tropical grass establishment in a given environment. They confirm the laboratory results in Section 4.1, which describes the ideal sowing time to maximise establishment as the “sowing window”. This optimises high establishment density, low weed burden, good persistence over summer and high autumn biomass. The sowing window is a compromise between sowing late enough so that soil temperatures do not limit germination and sowing early enough for sufficient root development prior to onset of the summer drought. These results suggest the sowing window at Gillingarra is from mid-August to early September. However, the sowing window varies with location and will be earlier for warmer and lower rainfall areas than for cooler, higher rainfall areas.

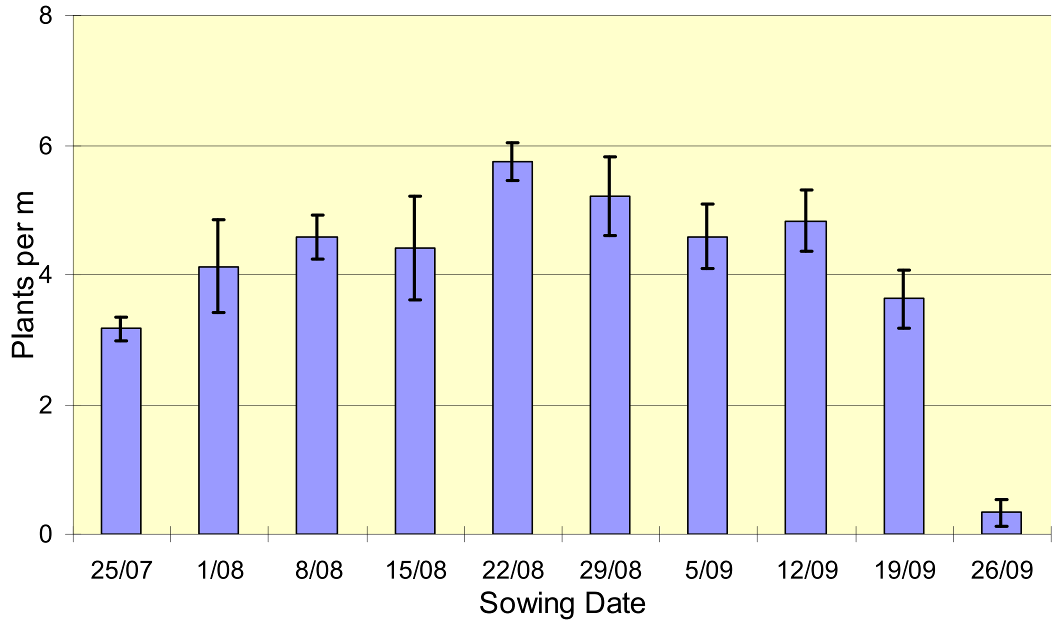


Figure 24. Panic grass counts (plants/m of row) on January 31, 2008 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors.

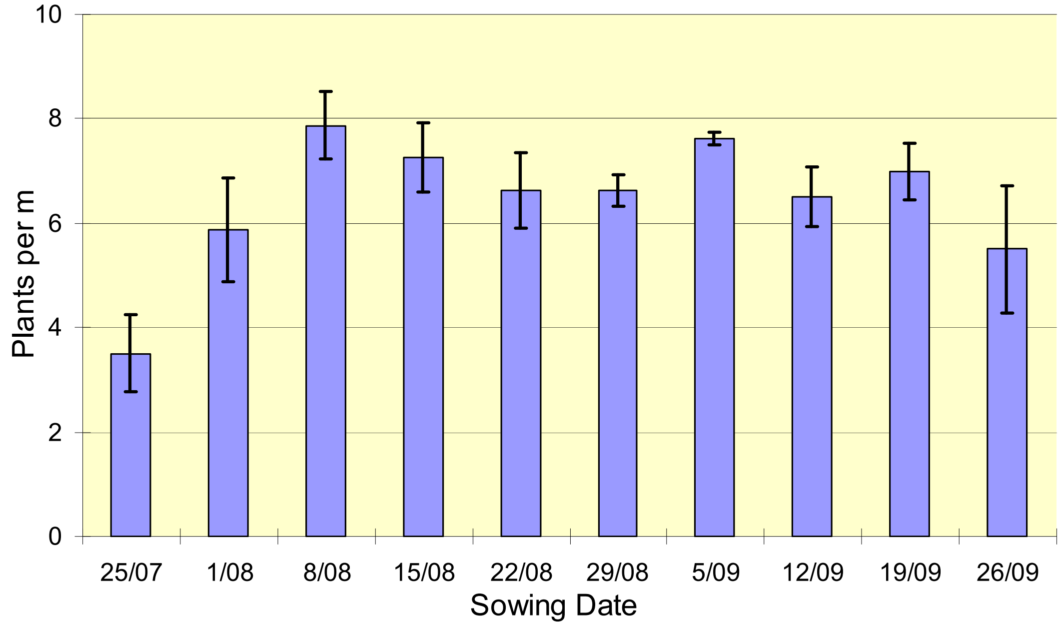


Figure 25. Rhodes grass counts (plants/m of row) on January 31, 2008 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors.

5.2. Sowing depth

Sowing too deep is the cause of establishment failure for many pasture species. Whereas, cereals can be sown up to 10 cm deep, most pasture species have much smaller seeds, and consequently have insufficient energy reserves to emerge from such depths. Sub-tropical grass seeds are very small. Lodge (G.M. Lodge, unpublished data) lists the following seed weights (uncoated) for some of the more widely sown sub-tropical grasses: Gatton panic grass (0.68 mg), Katambora Rhodes grass (0.24 mg), Premier digit grass (0.43 mg) and Bambatsi panic grass (0.93 mg). These compare with subterranean clover seed weights of 5-8 mg. Their small size indicates that sub-tropical grass seeds cannot be sown very deeply.

Lodge and Harden (2009) suggest that 10-25 mm is the ideal sowing depth for sub-tropical grasses in northern New South Wales. However, in their environment pastures are generally established in November-December, when soil temperatures are much warmer than the recommended time of August-September in WA. The soils in northern New South Wales also tend to be more fine-textured than the sandy soils typically sown to sub-tropical grasses in WA. An experiment was therefore conducted to investigate the optimum sowing depth for successful establishment on a typical soil type in WA.

Materials and methods

A micro-plot field trial was conducted at South Perth, WA (31°39’32”S, 115°53’14”E) to determine the optimum sowing depth for establishment of sub-tropical perennial grasses. The trial consisted of five perennial grass species (Gatton panic, Katambora Rhodes grass, signal grass, Whittet kikuyu, and Splenda setaria). The small-seeded perennial legume (*Lotononis bainesii* cv. Miles) was also sown. The site consisted of a sandy soil, which had been cultivated, smoothed to make a level surface and sprayed with glyphosate at 2L/ha for weed control two weeks prior to sowing.

Two sowing dates were used (24 August and 28 September) of 2006. Plots comprised rows of 400 mm length with furrows formed to make six sowing depths: 5 mm, 10mm, 15mm, 20mm, and 30mm and surface-sown. The trial consisted of four replicates arranged in a randomised complete block design.

Furrows were formed by carefully tapping wood blocks with dowelling of the appropriate diameter (see Figures 25 and 27). In each row 100 germinable seeds (identified as containing embryos using the Kings Park Faxitron MX-20 x-ray machine) were evenly spread and the furrow back-filled with sand to surface level. Plots were irrigated daily by overhead sprinklers.

Emerged seedlings were counted weekly for 28 days. Two randomly chosen seedlings per plot were cut to ground level on December 2 for measurement of dry weight.

Figure 26. Forming rows of different depths at South Perth, WA.

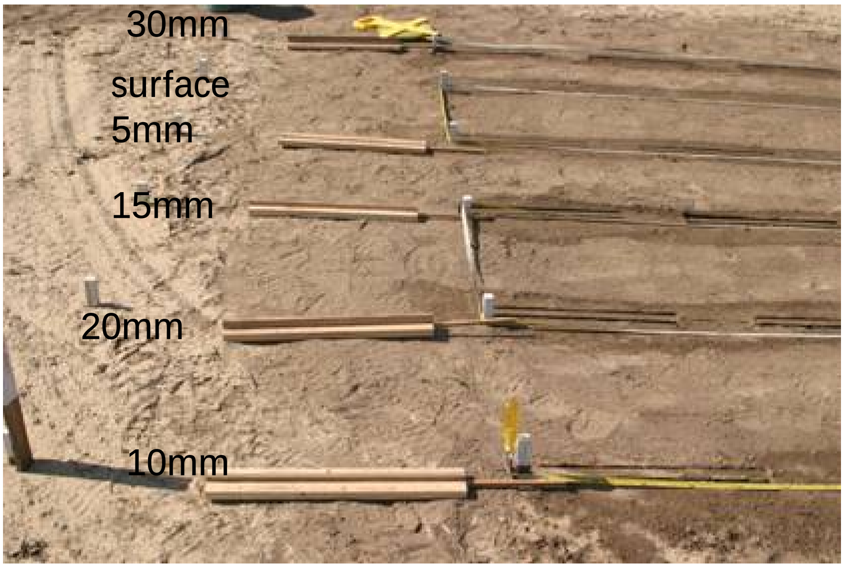


Figure 27. Blocks with dowelling used to form rows of different depths at South Perth, WA.

Results and discussion

Mean monthly maximum and minimum temperatures for the South Perth site for July –December, 2006 are shown in Figure 28.

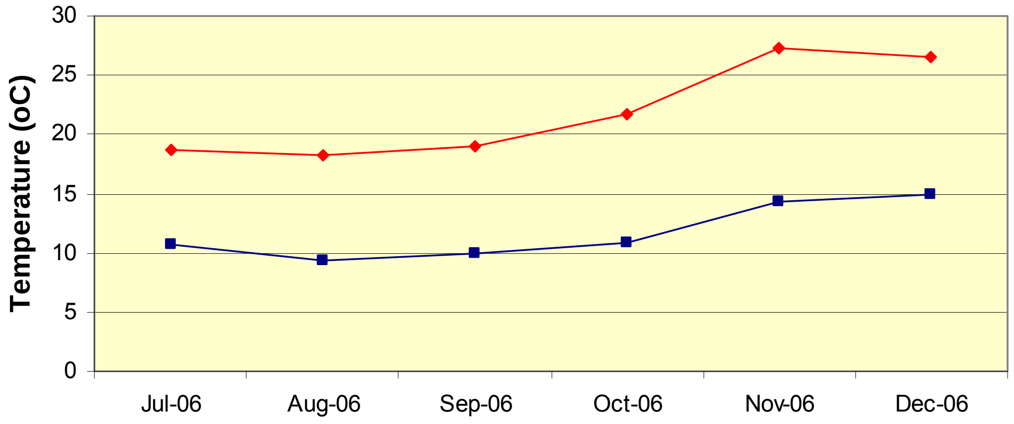


Figure 28. Mean monthly maximum (red) and minimum (blue) temperatures from July 1 to December 31, 2006 at South Perth, WA (data from the DAFWA Client & Resource Information System database).

Time of sowing (TOS), when averaged across all six species, did not have a significant effect on seedling emergence under the sowing conditions at South Perth and there was no interaction between sowing depth and TOS (Figure 29). Therefore, data from both TOS were combined in further analyses.

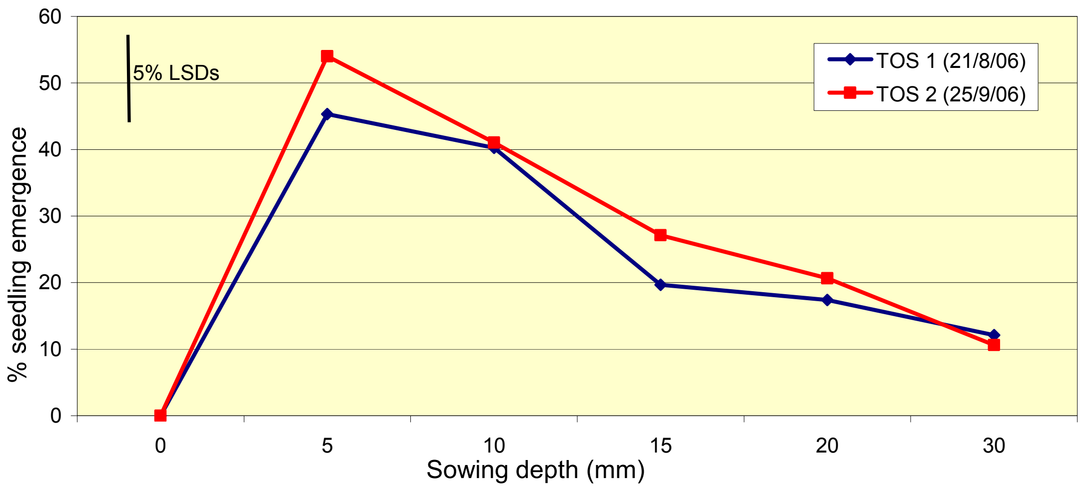


Figure 29. Mean seedling emergence (% of seeds sown) of six sub-tropical perennial species at six different sowing depths from two times of sowing (TOS) at South Perth, WA.

The important finding from this experiment is that a single sowing depth of 5-10 mm appears suitable for each of the species studied. Seed sown on the surface of all species failed to establish, while seed of all species established well from 5 mm and 10 mm sowing depth (Figure 30). The smaller-seeded species, notably Rhodes grass and *Lotononis bainsii*, had major reductions in establishment density at sowing depths deeper than 10 mm, while the largest-seeded species, signal grass, was able to establish from deeper sowings.

The optimum sowing depth for sub-tropical grasses found in this experiment is shallower than the 10-25 mm optimum suggested by Lodge and Harden (2009) for northern New South Wales. This may be due to slower germination and early seedling growth due to temperatures during the August-September establishment period in Perth being much cooler than those in the November-December establishment period of northern New South Wales. There may also be a soil texture factor, with the soils in northern New South Wales tending to be more fine-textured than the sandy soils typically sown to sub-tropical grasses in WA.

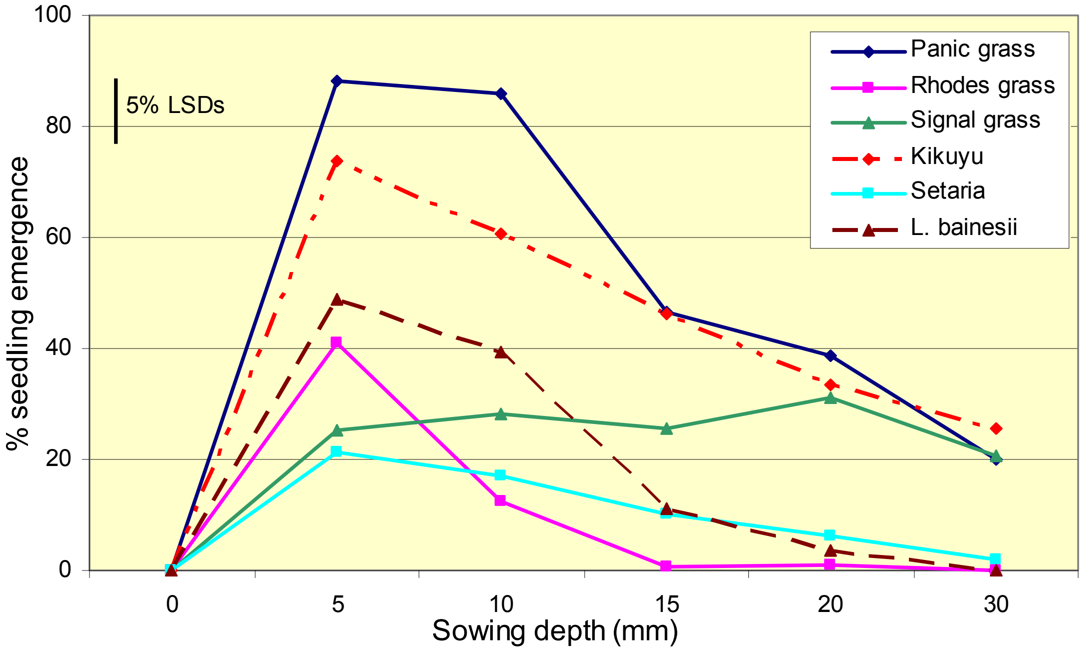


Figure 30. Seedling emergence (% of seeds sown) of six sub-tropical perennial species at six different sowing depths (mean of two times of sowing) at South Perth, WA.

5.3. Pre-sowing weed control strategies

Producer experience in WA has shown it is essential to sow sub-tropical perennial grasses into a weed-free seed bed, as their seedlings are weak competitors with both established winter growing pasture plants and newly germinated summer growing weeds (Moore *et al.* 2006). Lodge *et al.* (2010) also demonstrated the need for good pre-sowing weed control in northern New South Wales.

At the commencement of the project, a common method of pre-sowing weed control was to use two knockdown herbicides, 5-6 weeks before sowing and again at 2 weeks before sowing. While this generally prepares a clean, weed-free seed bed, a potential problem is that it leaves sandy soils very prone to wind erosion during spring. A series of experiments were conducted to look at different pre-emergent knockdown options.

5.3.1. The need for good weed control

An experiment was conducted near Geraldton to test whether a single knockdown herbicide application just prior to sowing could be used as a tactic to reduce the chances of wind erosion on erosion-prone soils. This was compared with the double knockdown strategy. However, the single knockdown treatment only resulted in partial weed control, particularly of erodium (*Erodium cicutarium*), which was little affected by the herbicide. The aim of the trial was then changed to compare sub-tropical grass establishment following good versus poor weed control.

Materials and methods

The trial was located on a grey sandy soil at Kojarena, 28 km east of Geraldton (28°42’01”S, 114°53’07”E). Soil test information for the surface 10 cm of soil is shown in Table 14.

Ammonium Nitrogen	Nitrate Nitrogen	Phosphorus Colwell	Potassium Colwell	Sulphur	Organic Carbon	Conductivity $EC_{1:5}$	pH ($CaCl_2$)
mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	dS/m	pH
2	10	14	83	48.90	0.92	0.150	5.40

Table 14. Soil test information for the surface 10 cm at the Kojarena trial site. Analyses conducted by CSBP.

The botanical composition of the pasture at the time of initial spraying consisted largely of erodium, capeweed (*Arctotheca calendula*), annual ryegrass (*Lolium rigidum*) and barley grass (*Hordeum leporinum*), with a low density of subterranean clover (*Trifolium subterraneum*). Half the trial area was given an initial application of glyphosate (2L/ha, 450 g/L active ingredient) on July 16, 2009. The whole area was then sprayed with a mixture of glyphosate (1.5L/ha, 450 g/L active ingredient) with Dominex insecticide @ 100 mL/ha on August 20. The site was sown on August 25 to the Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass and 20% signal grass) using a Massey Ferguson combine seeder with scarifier openers, Soil rider® depth control that ensured sowing depth was 5-10 mm, and press wheels. Rows were 615 mm apart. Sowing rate was intended to be 5 kg/ha (with 40% germinability), but subsequent calculations showed it was 15 kg/ha. Four replications were used.

Plastic tags, 90 mm long with 300 mm spikes, were placed immediately following sowing in 9 random locations in the bottom of seeding furrows of each plot to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom.

The amount of sand infill into furrows was measured on these tags on September 30 (36 days after sowing) and December 2. Seedling counts were conducted in 1 m sections along the row either side of each tag on September 30, December 2 and July 14, 2010. Mean height of 6 seedlings in each 1 m section was measured on September 30. Biomass was measured on December 2, by cutting nine 1 m strips per plot to ground level and drying at 80°C for 48 hours.

Results and discussion

Soil moisture conditions were very favourable for germination and seedling growth. The soil surface was moist at sowing and 65 mm of rain fell in the following five weeks (Figure 31).

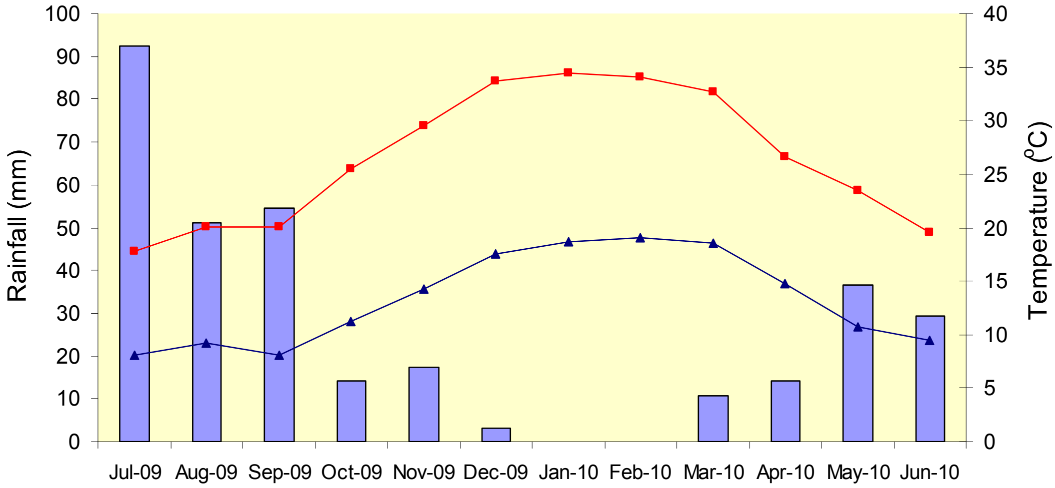


Figure 31. Monthly rainfall totals (bars) and mean maximum (red) and minimum (blue) temperatures from July 1, 2009 to June 30, 2010 at Northern Gully, WA, 6 km east of the Kojarena site (Data from the Australian Bureau of Meteorology).

Plant emergence in both treatments was extremely high (Table 15), in part from the excessive sowing rate and in part from excellent seasonal conditions for establishment. The double knockdown treatment produced significantly higher seedling densities 36 days after sowing than the single knockdown treatment (Figure 32), consistent with other trial findings at Gillingarra and Eneabba (see Section 5.3.2). It was noticeable that the seedbed was less cloddy in the double knockdown treatment, presumably because of greater breakdown of root residues, which resulted in more accurate seed placement. Seedlings were already much larger 36 days after sowing in the double knockdown treatment than in the single knockdown (Table 15). This was most likely due to the competition for moisture by the background plants that were only suppressed and not killed outright by the single glyphosate treatment.

By December 2, there was a large plant density decline in both treatments; density in the double knockdown treatment was only 16.5% of that 58 days after sowing, while the single knockdown had 18.5% of its prior density. However, plant density in the double knockdown plots was still significantly higher than in the single knockdown plots. Most tellingly, however, was the enormous size difference in the plants, with plants in the single knockdown plots having only 1.8% of the biomass of those in the double knockdown plots (Table 15). This is shown very clearly in Figure 33.

By July 14, 2010 (12 months after sowing), the double knockdown plots had six times the plant density of the single knockdown plots (Table 15). The mean density of 1.1 plant/m (equivalent to 1.8 plants/m^2) in the single knockdown plots would be widely regarded as a failed establishment. It is apparent that the smaller, weaker plants in these plots at the beginning of summer, failed to persist into the following autumn.

Table 15. Plant density, seedling height and biomass of sub-tropical perennial grasses (Evergreen Northern Mix) at Kojarena, following application of glyphosate (1.5L/ha) 5 days before sowing with (double knockdown) or without (single knockdown) glyphosate (2L/ha) application 5 weeks prior to sowing on August 25, 2009. Standard errors are shown in parentheses.

Treatment	Establishment density[1] (plants/m)			Seedling height[2] (mm)	Biomass[3] (grams)
	30/09/09	2/12/09	14/07/10	30/09/09	2/12/09
2 knockdowns	118.0 (6.6)	19.5 (2.6)	6.6 (0.3)	3.68 (0.34)	87.0 (1.2)
1 knockdown	78.3 (11.7)	14.5 (3.4)	1.1 (0.4)	1.33 (0.20)	1.6 (1.1)
Significance	$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$

[1]Measured on nine 2 m sections of row per plot
[2]Mean height of 6 seedlings in nine 1 m sections of row per plot
[3]Measured on nine 1 m strips per plot cut to ground level and dried at 80°C for 48 hours

Figure 32. Excellent establishment in the double knockdown treatment at Kojarena. Photograph taken on September 30, 2009 (36 days after sowing).

Single application of glyphosate five days before sowing

Double application of glyphosate

Figure 33. This shows the importance of good annual weed control for successful establishment and strong early growth of sub-tropical grasses. The front plot was sprayed with glyphosate (2 L/ha) 5 weeks and 5 days prior to sowing, while the rear plot was only sprayed 5 days prior to sowing. Photo taken at Kojarena on December 2, 2009, following sowing on August 25.

Sand infill into furrows was significantly less (approximately half) in the single knockdown plots than the double knockdown, when measured 58 days and 130 days after sowing (Table 16). This indicates the additional biomass of the single knockdown treatment at the time of sowing was able to limit sand movement. However, it was not possible to test whether this strategy was successful in limiting soil erosion on a paddock scale, as no erosion-causing winds occurred during the establishment phase. However, it is apparent that herbicide strategies that leave more above ground biomass below ground root mass just prior to sowing have the potential to reduce the risks of soil movement on erosion-prone soils. However, this could mean accepting lower plant numbers.

Table 16. Infill of sand (mm) into furrows sown with sub-tropical perennial grasses (Evergreen Northern Mix) at Kojarena, following application of glyphosate (1.5L/ha) 5 days before sowing with (double knockdown) or without (single knockdown) glyphosate (2L/ha) application 5 weeks prior to sowing on July 25, 2009. Standard errors are shown in parentheses.

Treatment	Date measured[1]	
	30/09/09	2/12/09
2 knockdowns	11.4 (1.1)	17.6 (1.2)
1 knockdown	5.4 (0.6)	8.2 (1.1)
Significance	$P < 0.001$	$P < 0.001$

[1]Changes in sand height measured on plastic tags placed in the bottom of seeding furrows (9 per plot)

5.3.2. Further comparisons with one or two knockdown herbicides

The trial at Kojarena described in Section 5.3.1 was not a good test of the possible use of a single knockdown herbicide strategy, as the single knockdown treatment was not adequate to kill all plants in the existing pasture (particularly erodium). Its application only 5 days before sowing also resulted in a cloddy seedbed, as root residues had not had the opportunity to break down. The double application is widely regarded as the best paddock preparation for weed control, but it leaves the paddock at high risk of wind erosion, particularly on sandy soils in exposed paddocks. Two other trials were conducted at Gillingarra and Eneabba to compare the use of a single knockdown herbicide application with a double application. The aim was to determine whether the single knockdown strategy can minimise the potential for wind erosion on sandy soils during the seeding and establishment phase, without a major reduction in plant establishment.

Materials and methods

The Eneabba site was located 14 km south-east of Eneabba (29°55'54"S, 115°12'37"E) while the Gillingarra site was located 35 km south of Moora, WA. The soil type at both sites was a non-wetting grey sand. Further details for the surface 10 cm of soil are provided in Table 17.

Site	Ammonium Nitrogen	Nitrate Nitrogen	Phosphorus Colwell	Potassium Colwell	Sulphur	Organic Carbon	Conductivity $EC_{1:5}$	pH ($CaCl_2$)
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	dS/m	pH
Eneabba	3	13	9	43	55.30	1.45	0.089	5.40
Gillingarra	3	57	28	87	173.00	1.64	0.297	5.50

Table 17. Soil test information for the surface 10 cm at the Eneabba and Gillingarra trial sites. Analyses conducted by CSBP.

At both sites an area of 48 m x 48 m was sown to the Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass and 20% signal grass) @ 5 kg/ha (40% germinability) using an experimental cone seeder with scarifier openers, Soil rider® depth control that ensured sowing depth was 5-10 mm, and press wheels. Rows were sown 550 mm apart.

The area was sprayed in 6 m widths alternatively with one or two knockdown sprays (4 replicates). The first knockdown herbicide (glyphosate @ 2 L/ha, 450 g/L active ingredient) was sprayed 6 weeks before sowing and the second (glyphosate @ 1.5 L/ha, 450 g/L active ingredient) was sprayed on August 19 (2 weeks before sowing) (Figure 34). Dominex insecticide @ 100 mL/ha was also mixed with the second herbicide application.

Eight 90 mm long plastic tags with 300 mm spikes were randomly placed in the bottom of seeding furrows in each plot immediately after sowing (64 tags in total) to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom.

The amount of sand infill into furrows was measured on these tags 29 days after sowing. Seedling counts were also conducted in 1 m sections along the row of each tag.

Figure 34. Pre-emergent herbicide demonstration trial at Eneabba, showing strips with one or two applications of glyphosate. Photograph taken on August 21, 2009, immediately prior to the second herbicide application.

Results and discussion

Soil moisture conditions were favourable for germination and seedling growth at both sites. The soil surface was moist at sowing and 44 mm of rain fell in the following five weeks at Gillingarra (Figure 35) and 37 mm of rain fell at Eneabba (Figure 36).

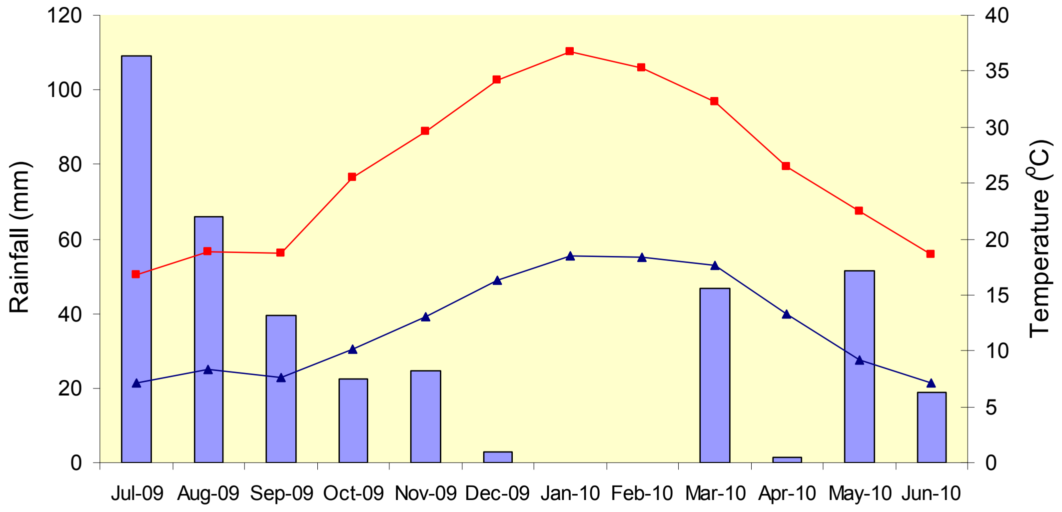


Figure 35. Monthly rainfall totals (bars) and mean maximum (red) and minimum (blue) temperatures from July 1, 2009 to June 30, 2010 at Moora, WA, 20 km north of the Gillingarra site (Data from the Australian Bureau of Meteorology).

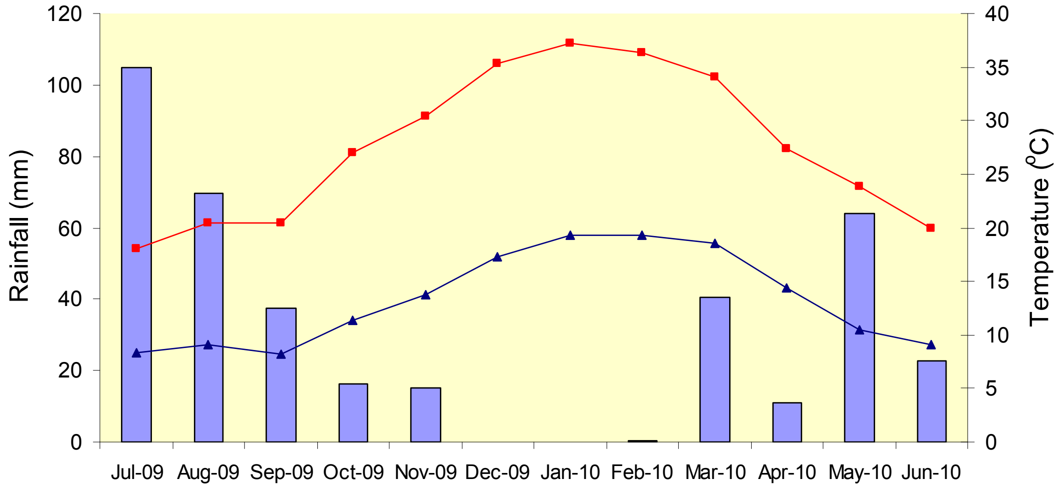


Figure 36. Monthly rainfall totals (bars) and mean maximum (red) and minimum (blue) temperatures from July 1, 2009 to June 30, 2010 at Eneabba, WA (Data from the Australian Bureau of Meteorology).

Establishment densities were higher following the double knockdown treatment at both sites (Tables 18 and 19), consistent with the findings at Kojarena (Section 5.3.1). Sand infill into furrows following sowing was again less with the single knockdown treatment, but unlike the Kojarena site, these differences were not statistically significant (Table 18 and 19).

The higher establishment density from the double knockdown pre-emergent herbicide regime presumably results from more accurate seed placement, due to the greater breakdown of soil organic matter. Indeed, it was noticeable that the double knockdown treatment at each site resulted in a very clean, weed-free seedbed with little plant residue, while the single knockdown treatment resulted in a more cloddy seed bed, due to the soil being held together by undecomposed roots. However, these results need to be treated with some caution. Spring conditions in 2009 were highly favourable and the soil surface remained moist at each site during pasture establishment and was not subjected to a major wind erosion event. Thus, this was not a true test of the ability of a single knockdown treatment to reduce wind erosion.

Another factor to be considered in drier winters and springs, is that a single late knockdown will leave less soil water available to newly sown perennial grasses, which may impact on establishment success. The results from Kojarena showed this.

The conclusion from these trials is that although a single knockdown treatment results in lower establishment density, plant numbers can still be adequate if there is a good weed kill prior to sowing.

Table 18. Establishment density and seed infill into sowing furrows of the Evergreen Northern Mix, following one or two pre-emergent knockdown herbicide applications at Gillingarra

Treatment	Establishment (plants/m^2)	Sand infill into furrows (mm)
One knockdown	26.0	8.6
Two knockdowns	42.9	10.6
Significance	$P < 0.05$	Not significant

Table 19. Establishment density and seed infill into sowing furrows of the Evergreen Northern Mix, following one or two pre-emergent knockdown herbicide applications at Eneabba

Treatment	Establishment (plants/m^2)	Sand infill into furrows (mm)
One knockdown	8.0	7.6
Two knockdowns	16.8	7.4
Significance	$P < 0.001$	Not significant

5.3.3. Fine-tuning weed control strategies

Several producers have developed other strategies for pre-emergent weed control, with the aim of obtaining a good weed kill prior to sowing while reducing the erosion risk of the paddock during the seeding and establishment phase. One that is becoming more widely used involves a selective broadleaf herbicide targeting ‘difficult to control weeds’ followed by a knockdown herbicide. Annual grasses are retained following the broadleaf herbicide application, which enables their continued root growth to bind the soil together until the knockdown herbicide application.

Materials and methods

In order to compare the effectiveness of different pre-emergent herbicide treatments, an experiment was conducted in 2009 in conjunction with the Evergreen Farming group on a deep, non-wetting sand at Gillingarra. Three treatments were compared:

a) A single knockdown sprayed on August 15 (glyphosate @ 1.5 L/ha);

b) A double knockdown sprayed on July 22 (glyphosate @ 2 L/ha) and August 15 (glyphosate @ 1.5 L/ha); and

c) 2-4,D ester @ 700 mL/ha for broadleaf control on July 22, followed by glyphosate @ 1.5 L/ha on August 15

Formulation of the glyphosate was 450 g/L active ingredient, while the 2-4,D ester was 800 g/L active ingredient.

Plot areas were 90 m long x 10 m wide and the trial consisted of a randomised block design with three replicates. The Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) was sown @ 4 kg/ha (40% germination) on August 27 across all plots.

Sowing was conducted perpendicular to spray treatments by three different seeding machines, each in sub-plots of 150 m length x 40 m width. The three machines were a Chamberlain combine, an International combine and Massey Ferguson combine. These machines are discussed in more depth in Section 6.

Nine 90 mm long plastic tags with 300 mm spikes were randomly placed in the bottom of seeding furrows in each plot 5 days after sowing to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom.

Seedling counts and sand in-fill were measured 47 days after sowing.

Results and discussion

Soil moisture conditions were favourable for germination and seedling growth. The soil surface was moist at sowing and 44 mm of rain fell in the following five weeks (Figure 35). The double knockdown herbicide treatment had the highest overall seedling establishment (Table 20), consistent with other studies. Examination of the seedbed showed it to be less cloddy than the other herbicide treatments across the site, which presumably resulted in more accurate seed placement. However, the selective broadleaf treatment still gave a high establishment density, which was significantly higher than the single knockdown treatment (Table 20). Individual machinery effects are discussed in Section 6.

Sand in-fill into furrows was highest overall for the double knockdown treatment and was similar for the other two treatments (Table 20). Had severe wind events occurred in the 4 weeks after sowing, it is likely that establishment in the double knockdown treatment would have been adversely affected.

Table 20. Establishment density (plants/m^2) 47 days after sowing of perennial grasses (Evergreen mix) and sand in-fill into furrows, following three different pre-emergent herbicide treatments on a loose, sandy soil at Gillingarra, WA.

	Pre-emergent herbicide treatment		
	1 knockdown	2 knockdowns	Broadleaf selective then knockdown
Plant density (plants/m^2)	13.6	27.2	21.0
l.s.d. (P = 0.05)	5.4		
Sand in-fill (mm)	6.4	12.0	8.8
l.s.d. (P = 0.05)	2.5		

These results suggests a broadleaf selective herbicide, followed by a knockdown herbicide, is a good compromise between the requirement for a weed-free seedbed and reduced erosion potential.

5.4. Seeding machinery design

5.4.1. Tyne point configuration

A trial was sown to determine the most appropriate tyne-point configuration for the establishment of Gatton panic and Katambora Rhodes grasses. The trial attempted to investigate the importance of depth wheels for accurate seed placement and whether non-wetting granules increase establishment when using t-boots. Additionally, the effect of sand infill into sowing furrows was measured.

Materials and methods

The trial was sown on a non-wetting grey sand at Gillingarra, WA. Further details for the surface 10 cm of soil are provided in Table 17. An experimental cone seeder was used to sow 12 different tyne treatments (Table 15), replicated four times and arranged in a randomised block design. The following tyne points were used:

i) t-boot seeding knife point (TKP);

ii) half 8”-wide scarifying points (½HWP);

iii) a modified 8”-wide scarifying point (WP); and

iv) a modified 8”wide scarifying point minus the end point (WPcut).

Table 15. Tyne-point configurations for the establishment of panic and Rhodes grasses at Gillingarra, WA.

Treatment	Sowing Configuration
TKP–	t-boot knife points with no depth wheel
TKP+	t-boot knife points with depth wheel
TKPNW–	t-boot knife points + non-wetting granules with no depth wheel
TKPNW+	t-boot knife points + non-wetting granules with depth wheel
½WP–	Half wide point (8 inch) with no depth wheel
½WP+	Half wide point (8 inch) with depth wheel
WP–	Wide scarifying point (8 inch) with no depth wheel
WP+	Wide scarifying point (8 inch) with depth wheel
WPcut–	Wide scarifying point - no tip (8 inch) with no depth wheel
WPcut+	Wide scarifying point - no tip (8 inch) with depth wheel
WPang–	Wide scarifying point (8 inch) on angle with no depth wheel
WPang+	Wide scarifying point (8 inch) on angle with depth wheel

DW= depth wheel

Prior studies and anecdotal information from farmers indicated the need for press wheels for good seed contact with soil moisture, particularly on sandy soils. Consequently press wheels were used for all treatments. Each configuration was tested with or without depth wheels, that act to control sowing depth, to determine the importance of sowing depth precision. For treatments WPang– and WPang+, the tyne was placed on a raised angle. Non-wetting granules were also tested with t-boot knife points (treatments TKPNW– and TKPNW+), as a possible means of overcoming non-wettability of the soil.

Seed was sown on August 22, 2007 to a depth of 5 mm into plots consisting of three 6 m rows, spaced 0.55 m apart, with 2 m buffers between plots. Gatton panic was sown in the outer two rows, while Katambora Rhodes grass was sown in the central row. Sowing rates were adjusted to give a target of 50 germinable seeds per metre (identified as containing embryos using the Kings Park Faxitron MX-20 x-ray machine).

Three 90 mm long plastic tags with 300 mm spikes were randomly placed in the bottom of seeding furrows in each row immediately after sowing to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom.

Plant counts were conducted on emerged seedlings on October 3 (43 days after sowing) and on January 31, 2008. Sand infill into furrow bottoms was also measured on October 3.

Figure 37. Ron Yates demonstrating seeding tyne configurations to producers at Gillingarra in November 2007

Results and discussion

Monthly rainfall totals and mean maximum and minimum temperatures from July 1, 2007 to March 31, 2008 are shown in Figure 18, while soil temperatures are shown in Table 11. The site received approximately 130 mm rainfall from July to September, which resulted in good soil moisture conditions for germination and establishment.

Depth wheels resulted in greater seedling emergence, particularly for the small-seeded Rhodes grass (Figures 38 and 39). This was due to the action of ensuring seed is placed precisely to the intended depth. This greater precision made a large difference to establishment success and confirms the importance of precise sowing of perennial grasses to a depth of 5-10 mm. The non-wetting granules did not increase seedling counts.

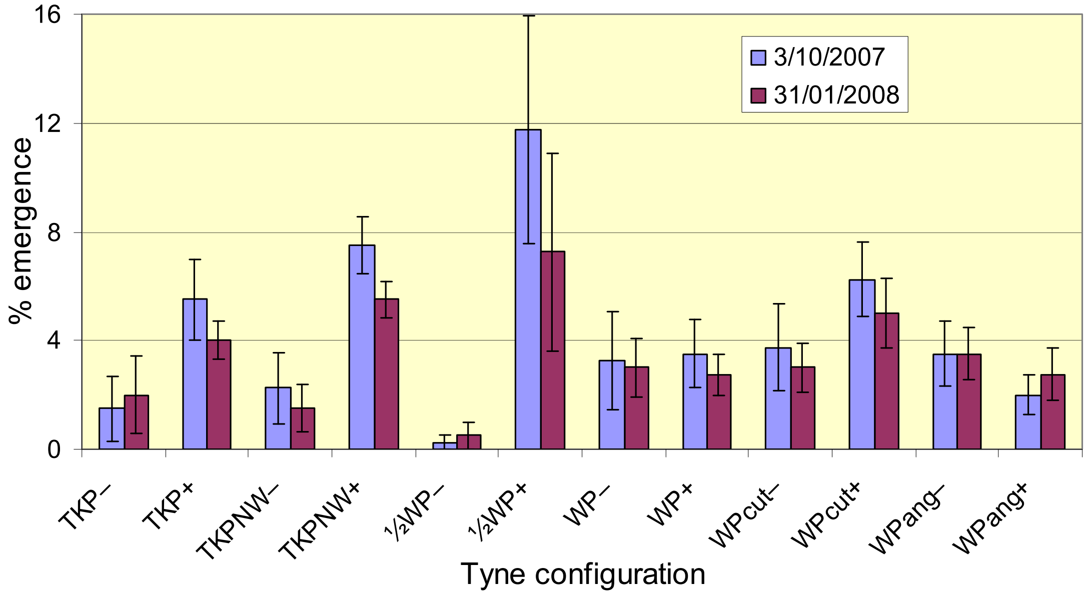


Figure 38. Plant densities (percent of germinable seeds sown) of Rhodes grass on October 3, 2007 and January 31, 2008, following sowing with twelve different seeding configurations (see Table 15 for code descriptions) at Gillingarra, WA. Plots sown on August 22, 2007. Bars shoe standard errors.

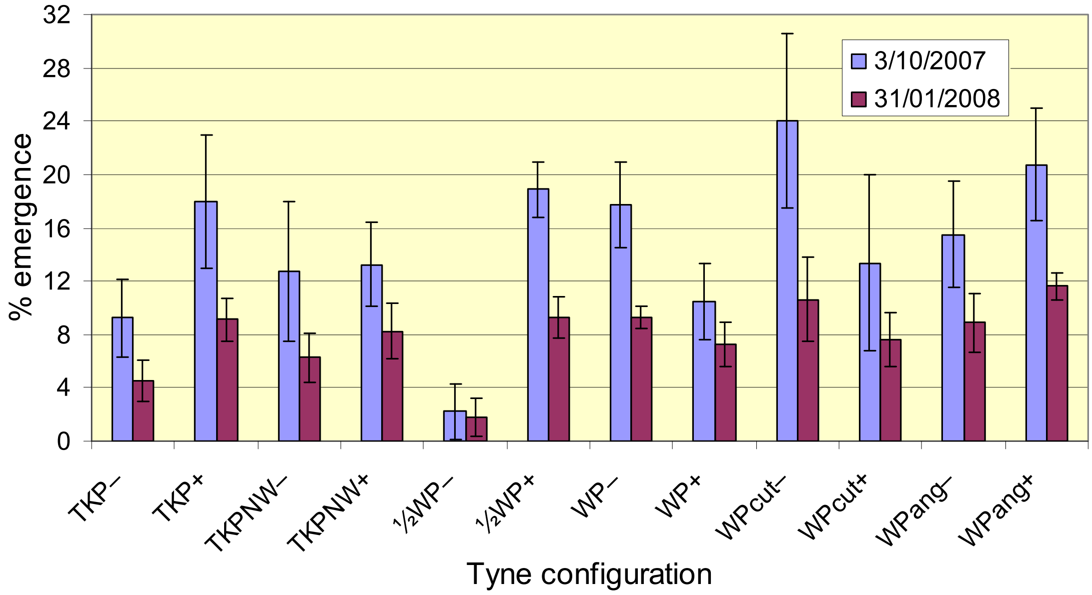


Figure 39. Plant densities (percent of germinable seeds sown) of panic grass on October 3, 2007 and January 31, 2008, following sowing with twelve different seeding configurations (see Table 15 for code descriptions) at Gillingarra, WA. Plots sown on August 22, 2007. Bars shoe standard errors.

The best sowing configuration for Rhodes grass was treatment ½WP+, which resulted in 12% emergence of all seeds sowed 43 days after sowing (Figure 38). For panic grass, the highest emergence counts came from treatments WPcut– and WPang+, with 19% and 24% emergence of all seeds sowed, respectively (Figure 39).

Table 16. Sand infill (mm) into furrows following sowing of panic and Rhodes grasses with twelve different tyne-point configurations (see Table 15 for code descriptions) at Gillingarra, WA.

Treatment	Sand infill (mm)
TKP–	11
TKP+	4
TKPNW–	10
TKPNW+	5
½WP–	23
½WP+	14
WP–	17
WP+	29
WPcut–	2
WPcut+	9
WPang–	12
WPang+	19

5.4.2. Furrow design

Furrows are generally used for sowing crops and pastures. They harvest moisture by increasing the available surface area able to intercept rainfall and channel it to the furrow bottoms where seeds are located. Thus, small rainfall events are more effective for plant growth than would be the case on a flat soil surface. Effective harvesting of rainfall is likely to be of particular importance to sub-tropical perennial grasses sown in south-western Australia, as soil moisture rapidly declines during spring and any out-of-season rainfall needs to be captured and made use of by developing seedlings. The importance of shallow sowing depth for reliable establishment of sub-tropical perennial grasses was clearly demonstrated in Section 5.2. It was also found that dropping seed onto the soil surface with no incorporation gave very poor establishment. It was important to check whether these findings from small hand-sown irrigated plots could be transferred to machinery-sown plots in target environments.

A series of experiments was conducted to compare establishment success in different furrow designs. Sowing into furrows was compared with sowing on the surface. The effectiveness of press wheels to push the seed below the soil surface was compared with drilling a depth of 5-10 mm. Three different furrow depths were also compared, to determine whether deeper furrows could be used in drier seasons to sow into soil moisture at depth.

Materials and methods

Trials were sown in 2009 at Gillingarra, 35 km south of Moora (30°57’00”S, 115°59’25”E), Kojarena, 28 km east of Geraldton (28°42’01”S, 114°53’07”E), and 14 km south-east of Eneabba (29°55’54”S, 115°12’37”E). Site details for Kojarena are given in Table 14, and for Gillingarra and Eneabba in Table 17. Rainfall and temperature conditions from July 2009 to July 2010 are given in Figure 31 for Kojarena, Figure 35 for Gillingarra and Figure 36 for Eneabba.

Five seeding furrow designs were tested at each site. These were:

1. Drilling seed 5-10 mm below the surface into a 50 mm deep furrow;

2. Drilling seed 5-10 mm below the surface into a 100 mm deep furrow;
3. Drilling seed 5-10 mm below the surface into a 150 mm deep furrow;
4. Dropping seed onto the surface into a furrow; and
5. Dropping seed onto the surface with no furrow.

Press wheels were used in each case.

Sites were sprayed twice with glyphosate (450 g/L active ingredient) herbicide prior to sowing. The first application (2 L/ha,) was sprayed six weeks before sowing and the second (1.5 L/ha) was sprayed two weeks before sowing. Dominex insecticide @ 100 mL/ha was also mixed with the second herbicide application.

The Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) was sown @ 5 kg/ha (40% germination) using an experimental cone seeder with a half wide point (8 inch). Sowing depth in the first three treatments was controlled by depth wheels; these were removed when sowing the surface-sown treatments. Plots were 1.25 m x 30 m with rows 550 mm apart. Treatments were replicated three times in a randomised block design. Sowing dates were August 25 at Kojarena, September 1 at Gillingarra and September 2 for Eneabba.

Eight 90 mm long plastic tags with 300 mm spikes were randomly placed in the bottom of seeding furrows in each plot immediately after sowing to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom.

Initial seedling counts and sand infill measurements were conducted on September 30 at Kojarena, October 1 at Eneabba and October 13 at Gillingarra. Plant counts were conducted along seeding rows in 1 m quadrats placed against the plastic tags. Further plant counts and sand infill measurements were conducted in the same locations on December 1 at Kojarena, December 2 at Eneabba and December 22 at Gillingarra. Proportions of the sown species were also recorded. Plant counts to measure plant survival over the summer-autumn period following sowing were also made on the same plot locations. These measurements were made on July 13 at Gillingarra and July 15 at both Kojarena and Eneabba in 2010.

Analyses of Variance (ANOVA) was conducted on plot means to determine differences between treatments.

Results and discussion

Establishment conditions were very favourable at each site. The soil surface was moist at sowing and good follow up rains fell, particularly at Kojarena (see Figure 31), in the following five weeks.

There were no differences at Kojarena between furrow treatments for seedling counts or for plant survival over the summer-autumn period. This showed that the very favourable soil moisture conditions at this site during the establishment period were very forgiving and all methods under these conditions produced very high establishment densities and good plant survival over the summer-autumn period (Table 17).

Sowing onto the surface with no furrows gave significantly poorer establishment densities than all treatments sown into furrows at both Gillingarra (Table 18) and Eneabba (Table 19). This translated into markedly poorer plant survival over the following summer-autumn period; at Gillingarra plant density of this treatment in July was only 15% that of the best treatment (drilling seed 5-10 mm in a 50 mm furrow), while at Eneabba it was only 41%. These results demonstrate that sowing into furrows is far less risky than sowing directly onto the soil surface. In a dry spring it is expected that seedlings sown directly onto the soil surface would be at a greater disadvantage than those sown into furrows, due to a lack of water harvesting capability.

There were no significant differences in establishment counts at Gillingarra (Table 18) or Eneabba (Table 19) for plots sown into furrows 50 mm, 100 mm or 150 mm deep. There were also no significant differences in plant survival over the summer-autumn period after sowing. This indicates that deeper furrows could be successfully used in dry seasons, to enable sowing into soil moisture at depth. However, while not statistically significant at any site, there was a tendency for more sand in-fill into the bottom of furrows with increasing furrow depth (Table 20). This might be expected due to the greater surface area and roughness of deeper furrows. The potential advantage of deeper furrows in dry seasons could not be tested, due to the favourable soil moisture conditions for seedling establishment at each site.

Initial establishment densities at Gillingarra and Eneabba from seed dropped onto the bottom of furrows were less than that from seed drilled 5-10 mm deep. However, there were no significant differences in plant density between these treatments when measured in December or the following July. This indicates that drilling to a depth of 5-10 mm deep is the most reliable sowing method. However, for farmers without precision seeders, an acceptable method establishment method is to drop seed onto the bottom of furrows and allow press wheels to press the seed below the surface (approximating to a sowing depth of 5-10 mm).

Table 17. Plant counts on September 30 and December 22, 2009 and July 13, 2010 of sub-tropical perennial grasses (Evergreen Northern Mix) using five different seeding furrow treatments at Kojarena, WA. Plots sown on August 25, 2009.

Furrow treatment[1]	30/09/09	1/12/09				15/07/10
	Plant count (plants/m)	Plant count (plants/m)	Percentage composition (Rhodes grass)	(panic grass)	(signal grass)	Plant count (plants/m)
5-10 mm in a 50 mm furrow	50.0	13.7	24	70	5	7.2
5-10 mm in a 100 mm furrow	48.2	13.5	27	64	9	8.3
5-10 mm in a 150 mm furrow	57.3	13.4	21	73	6	8.0
Surface-sown into a 50 mm furrow	50.5	12.2	32	65	3	7.3
Surface-sown with no furrow	53.2	13.7	13	76	11	7.0
Significance	n.s.	n.s.				n.s.

[1]Note: All treatments used press wheels

Table 18. Plant counts on September 13 and December 22, 2009 and July 13, 2010 of sub-tropical perennial grasses (Evergreen Northern Mix) using five different seeding furrow treatments at Gillingarra, WA. Plots sown on September 1, 2009.

Furrow treatment[1]	13/10/09	22/12/09				13/07/10
	Plant count (plants/m)	Plant count (plants/m)	Percentage composition (Rhodes grass)	(panic grass)	(signal grass)	Plant count (plants/m)
5-10 mm in a 50 mm furrow	30.3	11.6	24.1	68.9	7.0	7.1
5-10 mm in a 100 mm furrow	25.8	9.4	24.2	68.7	7.1	5.9
5-10 mm in a 150 mm furrow	31.4	10.9	17.3	75.9	6.8	5.7
Surface-sown into a 50 mm furrow	18.7	9.7	26.0	71.4	2.6	5.7
Surface-sown with no furrow	12.0	2.7	15.9	73.7	10.3	1.1
Significance	$P < 0.01$	$P < 0.001$				$P < 0.001$
l.s.d. (P = 0.05)	6.33	2.35				1.47

[1]Note: All treatments used press wheels

Table 19. Plant counts on October 1 and December 1, 2009 and July 15, 2010

of sub-tropical perennial grasses (Evergreen Northern Mix) using five different seeding furrow treatments at Eneabba, WA. Plots sown on August 26, 2009.

Furrow treatment[1]	1/10/09	2/12/09				15/07/10
	Plant count (plants/m)	Plant count (plants/m)	Percentage composition (Rhodes grass)	(panic grass)	(signal grass)	Plant count (plants/m)
5-10 mm in a 50 mm furrow	26.3	9.4	26.2	65.4	8.4	6.7
5-10 mm in a 100 mm furrow	15.8	8.7	34.1	48.7	17.3	6.7
5-10 mm in a 150 mm furrow	21.2	10.1	15.2	65.9	18.9	4.3
Surface-sown into a 50 mm furrow	10.5	9.1	21.5	56.9	21.5	4.4
Surface-sown with no furrow	7.5	6.3	16.3	71.3	12.3	2.8
Significance	$P < 0.05$	n.s.				$P < 0.05$
l.s.d. (P = 0.05)	12.42					2.52

[1]Note: All treatments used press wheels

Table 20. Amount of sand in-fill (mm) in the bottom of furrows sown to sub-tropical perennial grasses (Evergreen Northern Mix) using five different seeding furrow treatments at Gillingarra, Eneabba and Kojarena, WA

Furrow treatment[1]	Gillingarra		Eneabba		Kojarena	
	13/10/09	22/12/09	1/10/09	2/12/09	30/09/09	1/12/09
5-10 mm in a 50 mm furrow	10.2	13.4	7.2	12.1	9.0	14.0
5-10 mm in a 100 mm furrow	11.8	16.2	8.8	15.8	13.3	19.0
5-10 mm in a 150 mm furrow	12.1	18.5	9.1	17.2	14.8	24.8
Surface-sown into a 50 mm furrow	10.0	12.9	6.7	11.8	9.3	13.8
Surface-sown with no furrow	6.1	6.9	4.8	6.2	6.5	7.0
Significance	n.s.	$P < 0.05$	n.s.	$P < 0.05$	$P < 0.05$	$P < 0.05$
l.s.d. (P = 0.05)		5.80		8.22	5.33	11.38

5.5. Sowing speed

Previous experiments have shown the need for precise sowing of sub-tropical perennial grasses to a depth of 5-10 mm. In order to sow precisely to this depth, logic suggests that calibration of sowing depth should be done at the intended sowing speed. A great temptation is to sow at a faster speed to get the job done more quickly, but this is likely to cause more soil disturbance and less accurate seed placement. An experiment was, therefore, set up to investigate the effect of sowing speed on establishment success of sub-tropical perennial grasses.

Materials and methods

The experiment was sown on September 1, 2009 at Gillingarra, 35 km south of Moora (30°57'00"S, 115°59'25"E). The soil type was a non-wetting grey sand. Further details for the surface 10 cm of soil are provided in Table 17. Rainfall and temperature conditions from July 2009 to July 2010 are given in Figure 35.

The area was sprayed twice with glyphosate (450 g/L active ingredient) herbicide prior to sowing. The first application (2 L/ha,) was sprayed on July 22 and the second (1.5 L/ha) was sprayed on August 15. Dominex insecticide @ 100 mL/ha was also mixed with the second herbicide application.

A Massey Ferguson combine seeder with scarifier openers, soil rider depth control and press wheels was set up to sow rows 615 mm apart. The Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) was sown @ 5 kg/ha (40% germination).

Calibration of a 5-10 mm sowing depth was made when towed by a tractor at 5 km/hr.

Plots were subsequently sown at 5 km/hr, 10 km/hr and 15 km/hr. Plots were 2.5 m (a seeder width) x 48 m long and treatments were replicated three times in a randomised block design. Seedling counts were conducted on September 13 and December 22, 2009. Further counts were to assess plant survival over the summer-autumn period were conducted on July 13, 2010. Plant counts were made in four random 1 m sections per plot, positioned along seeding rows.

Results and discussion

Sowing at 5 km/hr gave significantly higher seedling establishment densities 42 days after sowing than sowing at 10 km/hr and 15 km/hr (Table 21). However, by December 22, there were no differences between plots sown at 5 km/hr and 10 km/hr and plant survival over the summer-autumn period was no different between these two treatments. However, plots sown at 15 km/hr had significantly lower plant densities at each time of counting (Table 21). It was also noticeable that the 15 km/hr-sown plots had more soil disturbance.

These results indicate that tractor speed can impact on establishment success and that sowing should not be done at markedly higher speeds than the speed used to calibrate sowing depth. Sowing too fast can cause excessive soil movement, reducing the accuracy of seed placement. Sand in-fill increases and furrows can collapse, resulting in a deeper effective seeding depth and poor resulting establishment. Optimum speed is likely to vary with the type of machinery, but this experiment suggests speeds of 5-10 km/h are likely to give a good result. Some machinery modifications could be made to enable sowing speed to be increased. For example, some producers have added rubber guards to prevent soil from one furrow being thrown into adjacent furrows. As a guide, operators should calibrate sowing depth of 5-10 mm at normal operating speed and make adjustments if needed.

Table 21. Plant counts on September 13 and December 22, 2009 and July 13, 2010 of sub-tropical perennial grasses (Evergreen Northern Mix) following sowing at three different speeds at Gillingarra, WA. Plots sown on September 1, 2009.

Sowing speed	13/10/09	22/12/09				13/07/10
	Plant count (plants/m)	Plant count (plants/m)	Percentage composition (Rhodes grass)	(panic grass)	(signal grass)	Plant count (plants/m)
5 km/hr	26.4	11.3	16.3	72.8	10.9	8.2
10 km/hr	14.4	11.0	14.3	76.8	9.0	7.3
15 km/hr	6.6	4.4	10.5	82.8	6.7	2.8
Significance	$P < 0.05$	$P < 0.05$				$P < 0.01$
l.s.d. (P = 0.05)	11.31	5.65				3.10

5.6. Sowing rate of panic grass

The recommended sowing rate for sub-tropical grasses prior to this project was 4-5 kg/ha. This relatively high rate was based on the expectation that establishment may be poor or patchy. However, if best practice establishment agronomy is followed, this rate could be reduced, thereby saving seed costs, assuming seed with high levels of germination is sown. An experiment was conducted to measure establishment density of panic grass sown at different sowing rates.

Materials and methods

The experiment was sown on September 1, 2009 at Gillingarra, 35 km south of Moora (30°57'00"S, 115°59'25"E). The soil type was a non-wetting grey sand. Further details for the surface 10 cm of soil are provided in Table 17. Rainfall and temperature conditions from July 2009 to July 2010 are given in Figure 35.

A commercial seed lot of panic grass (cv. Gatton) with 40% germinability was used. Treatments comprised sowing rates of 2, 4 and 8 kg/ha of seed. An additional treatment used seed of 90% germinability sown at 2 kg/ha. Seed for this treatment came from the same seed lot as the other treatments. However, germination was enhanced by checking for seed fill, using a Faixtron X-ray machine, and checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

Plots were sown on September 1 using an experimental cone seeder with a half wide point (8 inch). Sowing depth in the first three treatments was controlled by depth wheels. Plot size was 5 m x 1.7 m, with a row spacing of 550 mm, and there were four replications of each treatment arranged in a Randomised Block design.

The area was sprayed twice with glyphosate (450 g/L active ingredient) herbicide prior to sowing. The first application (2 L/ha,) was sprayed on July 22 and the second (1.5 L/ha) was sprayed on August 15. Dominex insecticide @ 100 mL/ha was also mixed with the second herbicide application.

Plant counts were made on October 2 and December 22 of 2009 and on July 13, 2010. Analyses of Variance (ANOVA) was conducted on plot means to determine differences between treatments.

Results and discussion

There were no significant differences in establishment density on October 2 (35 days after sowing) in plots sown at 2 or 4 kg/ha (Table 22). Somewhat surprisingly, plots sown with seed screened to 90% germinability at 2 kg/ha had no higher initial establishment densities than those sown to unscreened seed at 2 kg/ha. However, sowing at 8 kg/ha resulted in a doubling of initial establishment density. Plant density declined considerably in all plots over the following 14 weeks (December 22). The largest decline (75%) was in plots sown at 8 kg/ha, but this rate still gave a significantly higher density than the other sowing rates (Table 22). Plant counts taken on July 2010, however, showed no differences in density from the different sowing rates.

Table 22. Plant counts on September 13 and December 22, 2009 and July 13, 2010 of uncoated Gatton panic grass seed sown at 2, 4, or 8 kg/ha (40% germinability) or 2 kg/ha screened to 90% germinability. Plots sown at Gillingarra on September 1, 2009.

Sowing rate	Plant count (plants/m)		
	2/10/09	22/12/09	13/07/10
2 kg/ha (90% germination)	19.7	5.7	5.3
2 kg/ha	18.1	5.6	5.0
4 kg/ha	23.9	6.6	6.2
8 kg/ha	39.3	9.8	5.1
Significance	P< 0.001	P<0.001	n.s.
l.s.d. (P = 0.05)	9.00	3.33	

These results indicate that a sowing rate of 2 kg/ha can give similar establishment results as higher sowing rates, provided seed is of reasonable quality (at least 40% germination) and that the other key factors affecting establishment are followed correctly.

5.7. Overcoming dormancy in panic grass

Section 4.3 showed that seed dormancy is an issue in panic grasses sown in WA using seed harvested during the previous summer-autumn period. Some seed companies are promoting the ability of compounds on their seed coatings to break this dormancy and increase seed germination. Experiments were, therefore, set up in the field and laboratory to compare a commercially available seed coating product for enhanced germination in panic grass with untreated seed and with other seed enabling treatments.

Materials and methods

Field experiment

The experiment was sown on September 1, 2009 at Gillingarra, 35 km south of Moora (30^{o}57’00”S, 115^{o}59’25”E). The soil type was a non-wetting grey sand. Further details for the surface 10 cm of soil are provided in Table 17. Rainfall and temperature conditions from July 2009 to July 2010 are given in Figure 35.

A commercial seed lot of panic grass (cv. Gatton) with 40% germinability was used. The following treatments were compared:

1. Uncoated seed primed with water (Primed H_2O);
2. Uncoated seed primed with the plant signalling compounds, ethylene (20 mM) and salicylic acid (0.5 mM) (Primed PSC);
3. Seed coated with a commercial dormancy breaking compound (Dormbreaker); and
4. Uncoated seed (untreated control).

Seed priming was conducted two weeks prior to seeding. Seed was then dried for 12 days in a 16^{o}C, 25% relative humidity room prior to sowing. The trial was sown at an equivalent rate of 2 kg/ha (adjusted to that of uncoated control seed).

Plots were sown on September 1 using an experimental cone seeder with a half wide point (8 inch). Sowing depth in the first three treatments was controlled by depth wheels. Plot size was 5 m x 1.7 m, with a row spacing of 550 mm, and there were four replications of each treatment arranged in a Randomised Block design. The area was sprayed twice with glyphosate (450 g/L active ingredient) herbicide prior to sowing. The first application (2 L/ha,) was sprayed on July 22 and the second (1.5 L/ha) was sprayed on August 15. Dominex insecticide @ 100 mL/ha was also mixed with the second herbicide application.

Plant counts were made on October 2 (35 days after sowing). Analyses of Variance (ANOVA) was conducted on plot means to determine differences between treatments.

Laboratory controls

Laboratory controls were run in parallel with the field trial. The same treatments were used as in the field trials, except that seeds were screened for 90% germinability by checking for seed fill, using a Faixtron X-ray machine, and checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

Four replicates of 25 seeds per treatment were plated into 90 mm Petri dishes containing a sand medium with 20% (w/w) moisture. This medium was used to ensure breakdown of the commercial coating product being examined as one of the treatments. Petri dishes were sealed with plastic to prevent evaporation and then incubated at an alternating 12/12 hour, 26/13^{o}C temperature regime in the dark for 30 days. Germination was scored every 4-5 days, with germinants removed from the Petri dishes. Germination rate index (GRI) was calculated according to the formulae in Section 4.4.1.

Results and discussion

Under laboratory conditions the commercial coating product gave no benefit to either germination percentage or GRI, whereas priming with both H_2O and plant signalling chemicals improved germination significantly (P < 0.001), compared with untreated seed (Figure 40). Under field conditions the commercial coating product produced significantly lower seedling emergence than untreated seed, but the chemical and water primed treatments did not produce significantly higher emergence than untreated seed (Figure 41).

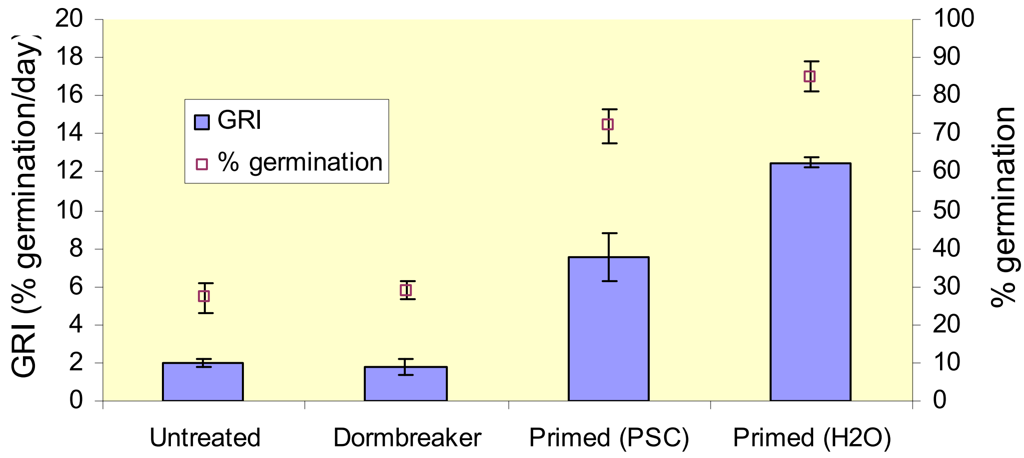


Figure 40. Percent germination and germination rate index (GRI) after 30 days in the laboratory of Gatton panic grass subjected to a range of seed enabling treatments and incubated in an alternating 26/13^{o}C, 12/12 hour light/dark regime. Bars represent standard errors.

Under the conditions of these experiments we were not able to show an advantage of the commercial coating product for enhancing germination. This may have been due to the use of seed with high germination; had seed been used with lower germination (and high seed dormancy) the opportunity would have been greater for expression of any benefits of the seed coating product. The higher gains in both germination percentage and GRI from chemical priming in the laboratory suggest the incorporation of dormancy breaking agents onto the seed coat may not be the best mode of action. This contrasts with chemical priming, where the plant signaling compounds are taken up directly by the seed prior to germination.

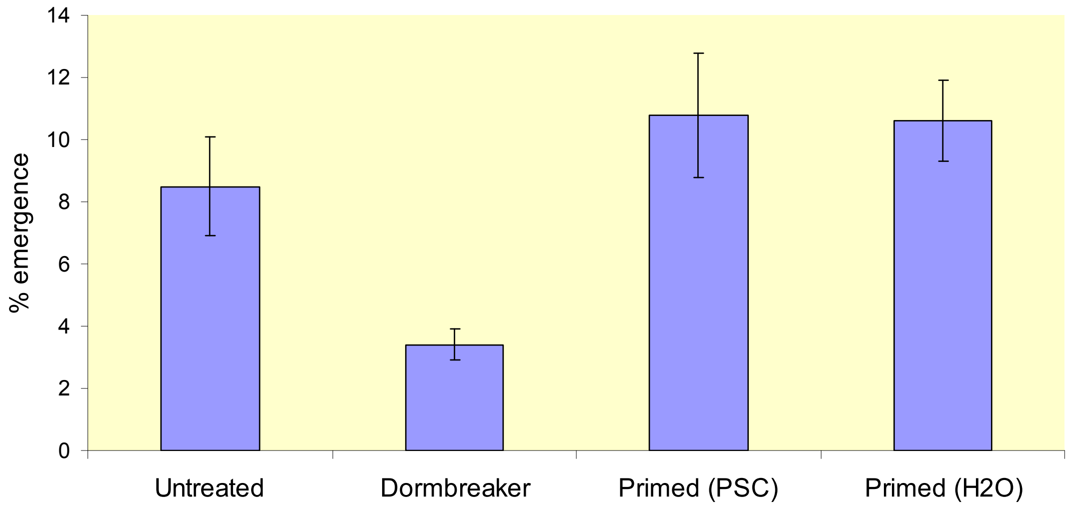


Figure 41. Seedling emergence 35 days after sowing of Gatton panic grass subjected to a range of seed enabling treatments and sown at an equivalent rate of 2 kg/ha in field plots at Gillingarra, WA on September 1, 2009. Bars represent standard errors.

These experiments showed the advantage of plant signaling chemicals in the laboratory were not transferred to the field, confirming observations in Section 4.4.4. These results suggest a greater understanding is needed of the biological and environmental factors that reduce this benefit under field conditions before they can be considered as a viable option by the seed industry.

5.8. Companion cereals for erosion control

The issue of wind erosion on exposed sandy paddocks during seeding and the establishment phase of sub-tropical perennial grasses is a major concern to growers in the NAR and south coast regions of WA. Section 5.3. discusses the strategic use of herbicides to maintain decaying root mass to help bind the soil at the time of sowing as a means of reducing erosion potential. Another possible option to help minimise wind erosion during the establishment phase is to sow a rapidly establishing cereal as a companion plant. A demonstration trial was sown to determine whether this is a feasible option.

Materials and methods

The trial was located on a grey sandy soil at Kojarena, 28 km east of Geraldton (28^{o}42’01”S, 114^{o}53’07”E). Soil test information for the surface 10 cm of soil is shown in Table 14. Rainfall and temperature conditions at the site are shown in Figure 31.

The Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) @ 5 kg/ha (40% germination) was sown on August 25 to a depth of 5-10 mm using an experimental cone seeder with half wide points (8 inch), depth wheels and press wheels.

Oats and sorghum were chosen as potential companion plants, oats being a winter-growing (C3) species, and sorghum being a summer-growing (C4) species. Seven treatments were compared, using four spacing arrangements:

1. Oats or sorghum every 9th row;
2. Oats or sorghum every 6th row;
3. Oats or sorghum mixed in the same row as the Evergreen mix; and
4. The Evergreen mix sown alone (control).

Cereal treatments were sown at an equivalent rate of 50 kg/ha in each row. Plots measured 20 m x 20 m, with 550 mm row spacings.

The trial area was given an initial application of glyphosate (2L/ha, 450 g/L active ingredient) on July 16, 2009, with a second application of glyphosate (1.5L/ha, 450 g/L active ingredient) on August 20. Dominex insecticide @ 100 mL/ha was mixed with the second application. The site was sown.

Nine plastic tags, 90 mm long with 300 mm spikes, were placed immediately following sowing in the bottom of seeding furrows of each plot to measure sand in-fill into furrows. These were positioned so that the lower edge of each tag was level with the furrow bottom. Tags were located in one of the cereal rows near the centre of each plot and in the four sowing furrows either side of this central tag. This was designed to examine the impact of the cereal row on sand infill with increasing distance from it. By having tags on either side of the cereal row, wind direction could be taken account of. For the control Evergreen mix and the mixed sowing plots, the same arrangement of tags was made around a central row near the centre of each plot.

Result and discussion

Rainfall conditions were very favourable for plant establishment (see Figure 31) and all plots established well (see Figure 42). The oat rows established more rapidly than the sorghum rows, which in turn established more rapidly than the C4 pasture grasses. The more rapid early growth of oats was not unexpected, as the soil temperatures from an August sowing date are cooler than optimum for the summer-active sorghum. A subsequent visit to the site in early December showed the sorghum rows growing rapidly, while the oats rows had senesced (Figure 42).

Data was not actively collected from the trial, as it was immediately apparent that neither the oats nor sorghum had the ability to grow fast enough during the first six weeks after sowing to have any impact on soil movement. There were no visual difference in establishment success and amount of sand infill in rows along transects either side of the companion cereal rows. However, the trial was not subjected to a major wind erosion event during the establishment phase, and so definitive conclusions about the merits of using a cereal to reduce sand movement cannot be drawn. Of the two species, oats is the better option. However, unless strong benefits can be shown, the additional hassle of setting up a seeding machine to sow separate rows of oats and the subsequent absence of perennial grasses in these rows means this option is unlikely to be widely adopted.

Figure 42. Photograph showing the cereal companion planting trial sown with the Evergreen mix at Kojarena on December 2, 2009. Sorghum can be seen actively growing in rows in the foreground plots (and in the immediate foreground), while oat rows in the background plots have senesced. The trial was sown on August 25, 2009.

6. Technology transfer to commercial machinery

Initial trials to develop the principles of an establishment package for sub-tropical perennial grasses were sown in small plots with an experimental cone seeder. However, for more general application, these principles needed to be tested using machinery commonly used by farmers. An old Massey Ferguson combine seeder (commonly found on many farms) was renovated and modified (Figures 43 and 44), using the information gained from the project, to sow larger-scale plot areas of perennial pastures and demonstrate the principles for good establishment.

A large demonstration site was subsequently organised with the Evergreen Farming Group to compare establishment success of sub-tropical perennial grasses using different commercial seeding equipment, but using the best practice principles developed in the project. The effects of different pre-emergent herbicide treatments on establishment success were also compared. These effects have already been discussed in Section 3.3.3.

Materials and methods

Three seeding machines were compared:

1. A farmer-operated Chamberlain combine with converted plough disc for scalping non wetting sand, a soil deflector and press wheels (row spacing 650 mm);
2. A farmer-operated International combine with standard knife points and press wheels (row spacing 180 mm); and

3. The DAFWA Massey Ferguson combine with scalping points, depth control by a local product called a Soil rider® and press wheels (row spacing 615 mm).

The trial was located at Gillingarra, 35 km south of Moora (30°57’00”S, 115°59’25”E) on a deep, non-wetting sand. Further details for the surface 10 cm of soil are provided in Table 17.

Figure 43. The renovated Massey Ferguson seeder adjusted to sow larger-scale paddock areas to perennial pastures.

Figure 44. Tyne configuration on the DAFWA Massey Ferguson seeder.

Plot areas of 150 m length x 40 m wide were sown by each machine on August 27 using the Evergreen mix @ 4 kg/ha. The majority of the site had a single knockdown herbicide spray on August 15 (glyphosate @ 1L/ha). However, 10 m wide pre-emergent herbicide treatment strips were sown perpendicular to the machinery treatments to determine whether a single knockdown herbicide application 2 weeks prior to sowing could be used on erosion-prone soils.

Herbicide treatments were replicated three times in a randomised block design and consisted of:

(i) A single knockdown sprayed on August 15 (glyphosate @ 1.5 L/ha);

(ii) A double knockdown sprayed on July 22 (glyphosate @ 2 L/ha) and August 15 (glyphosate @ 1.5 L/ha); and

(iii) 2-4,D ester @ 700 mL/ha for broadleaf control on July 22, followed by glyphosate @ 1.5 L/ha on August 15.

Formulation of the glyphosate was 450 g/L active ingredient, while the 2-4,D ester had 800 g/L active ingredient.

The layout of the trial is shown in Figure 45, while a photograph of the machines in operation is shown as Figure 46.

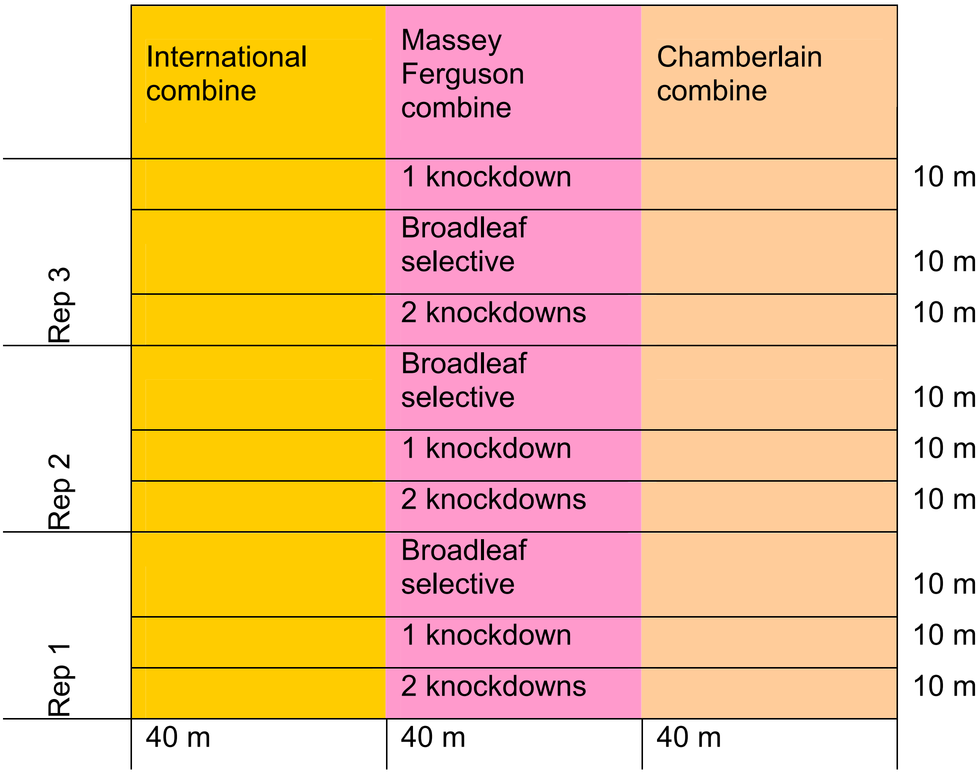


Figure 45. Machinery demonstration trial layout at Gillingarra sown in 40 m wide blocks to either an International combine, a Massey Ferguson combine or a Chamberlain combine, with perpendicular 10 m wide herbicide treatment strips.

Three plastic tags, 90 mm long, were placed in random locations in the bottom of seeding furrows of each machinery x herbicide combination treatment (total of 81 tags). The amount of sand infill into furrows was measured on these tags on October 2 (47 days after sowing). However, these were not placed until five days after sowing and so any initial sand infill was not measured. Seedling counts were also conducted in 1 m sections along the row either side of each tag.

Figure 46. Machinery comparison demonstration site at Gillingarra.

a. International combine, b. Chamberlain disc combine, c. Massey Ferguson combine (developed by the project), d. Farmers examining sown plots.

Results and discussion

Soil moisture conditions were favourable for germination and seedling growth. The soil surface was moist at sowing and 44 mm of rain fell in the following five weeks (Figure 35).

Mean establishment densities from the Massey Ferguson and International combine seeders were significantly greater than the Chamberlain disc seeder (Table 23). This was in spite of greater sand infill in the Massey Ferguson plots than the other two seeders (Table 24) during the measurement period. The reason for the lower plant density from the Chamberlain seeder may be due to deeper or less accurate seed placement. Observations at seeding showed that although this machine had the best scalping of non wetting sand, seeds sat on the scalped surface and the press wheels did not adequately cover them. It was also the most aggressive of the machines in terms of furrow development and soil movement. As sand infill was not measured in the first five days after sowing, it is possible that more initial infill occurred with this treatment. Although there were no significant differences between the Massey Ferguson and International combines, the later was visually patchier, with poor germination in some patches, possibly because the knife point tynes of the International seeder did not scalp the non wetting sand. The Massey Ferguson had some scalping of the non-wetting sand and had more accurate seed placement.

As discussed in Section 3.3.3., the double knockdown herbicide treatment resulted in significantly greater seedling establishment than the selective broadleaf treatment, which in turn was greater than the single knockdown treatment (Table 23). Examination of the seedbed showed the single knockdown treatment produced a cloddier seedbed, which presumably resulted in less accurate seed placement. This was not reflected in differences in the amount of sand infill into furrows (Table 24).

There was a significant interaction between seeding machinery and herbicide treatment, with the highest establishment being with the International seeder with two knockdowns and the lowest being for the Chamberlain seeder with a single knockdown spray (Table 23). However, the Massey Ferguson plots with a single knockdown still produced a higher plant density than the overall site mean.

These results indicate that good establishment appears to be possible using a range of different seeding systems, provided the key steps to establishment are followed. This is likely to over-ride machinery design. It also shows that use of a broadleaf herbicide, followed by a knockdown herbicide prior to sowing, can be used as a weed control strategy on soils prone to erosion, provided a seeding machine is used that can scalp away weeds and non-wetting sand, and sow to a shallow depth into furrows.

Table 23. Establishment density (plants/m^2) after sowing sub-tropical perennial grasses (Evergreen Northern Mix) with three different seeding machines and three different pre-emergent herbicide treatments at Gillingarra.

Seeding machine	Pre-emergent herbicide treatment			
	1 knockdown	2 knockdowns	Broadleaf selective	Mean
Massey-Ferguson	23.9	33.3	25.2	27.5
Chamberlain	7.1	9.1	9.3	8.5
International	9.9	39.2	28.4	25.8
Mean	13.6	27.2	21.0	20.6
Machinery effect	*P* < 0.001			
l.s.d.	5.48			
Herbicide effect	*P* < 0.001			
l.s.d.	5.48			
Machinery x herbicide interaction	*P* <0.01			
l.s.d.	9.49			

Table 24. Sand infill (mm) into sowing furrows 47 days after sowing sub-tropical perennial grasses (Evergreen Northern Mix) with three different seeding machines and three different pre-emergent herbicide treatments at Gillingarra.

Seeding machine	Pre-emergent herbicide treatment			
	1 knockdown	2 knockdowns	Broadleaf selective	Mean
Massey-Ferguson	6.4	12.0	8.8	9.1
Chamberlain	5.9	6.8	6.2	6.3
International	3.2	3.6	2.9	3.2
Mean	5.2	7.4	6.0	6.2
Machinery effect	*P* < 0.001			

l.s.d.	2.53
Herbicide effect	Not significant
Machinery x herbicide interaction	Not significant

7. An establishment package for sub-tropical perennial grasses

The results from this project, combined with data from the Evergreen group and the collective experiences of many leading farmers, has enabled us to formulate an agronomic package that will lead to reliable establishment of sub-tropical perennial grasses in south-western Australia.

A desirable benchmark for sub-tropical grass establishment is a density of 8-10 plants/m^2 surviving though to the first autumn after sowing. For a row spacing of 50-60 cm, this equates to 4-5 plants per metre in each seeding row. Small, weak plants in late November - early December are unlikely to persist over summer, unless there is good summer rainfall. On the other hand, plants which are strong and well established in late spring – early summer have a high likelihood of persisting over summer.

Bunch grasses, such as panic grass, setaria, digit grass and Bambatsi panic, do not spread by runners and rarely recruit new seedlings from seed. In general, the plant density of these species will not increase over time, although crowns of individual plants will get larger. Therefore, the medium to long-term plant density of the bunch grasses is largely determined by those plants that persist through to the end of the first autumn after sowing. The creeping grasses, such as Rhodes grass, kikuyu and signal grass, are more forgiving of poor establishment density, as they will spread and fill in gaps over time, given appropriate management.

The establishment package is based on ten steps and is presented below. It is relevant to Rhodes grass, panic grasses, Signal grass, Setaria, Digit grass, Bambatsi panic, kikuyu, Siratro and lotononis either sown alone or in mixtures. It is likely to also be relevant to other areas with Mediterranean-type climates. This package is summarised by Nichols *et al.* (2010) and presented in more detail by Moore *et al.* (2011*c*).

1. Plan ahead

Plan a year ahead and reduce weed seed-set by grazing and spray-topping, especially for difficult-to-control weeds. For exposed paddocks prone to wind erosion, consider sowing a cereal to provide stubble for soil stability. Commence control of rabbits and kangaroos if they are a potential problem.

2. Purchase good quality seed

Select species and varieties suited to your district, soil type and intended use. Refer to Moore *et al.* (2011*a*) for best sowing options for the northern agricultural region and to Moore *et al.* (2011*b*) for the south coast.

Germination of commercial sub-tropical grass seed batches typically varies from 10–60%. Kikuyu (commonly >80%) is an exception. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. Several seed companies coat their seeds. While this helps with handling and flow of fluffy seeds in machinery, it reduces the number of viable seeds per kilogram.

Seed dormancy

Panic grass, setaria and signal grass have a high proportion of seeds, referred to as *fresh seeds*, which remain dormant for 6–10 months after harvest. Consider purchasing these species the year before seeding and storing under dry conditions. Rhodes grass and kikuyu do not have such dormancy.

3. Control weeds and insects prior to sowing

A weed-free seed bed is essential as sub-tropical grass seedlings are weak competitors. Weed control strategies should aim to minimise wind erosion, particularly on exposed sites with sandy soils. One strategy is to use a selective broadleaf herbicide 6 weeks from sowing, followed by a general knockdown herbicide 2 weeks from sowing. This allows grass residues to bind the soil and reduce erosion risk.

In paddocks with a low grass density, a single knockdown herbicide 2 weeks before sowing can be used, but higher rates than autumn-winter applications will be needed to kill difficult-to-control weeds.

Two knockdown sprays (6 weeks and 2 weeks before seeding) result in good weed control but leave the paddock prone to wind erosion (unless stubbles have been retained). It also reduces the amount of winter grazing and the ability to change plans if sowing conditions deteriorate.

Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

4. Sow into moisture in late winter – early spring

The best sowing times for the main regions suited to sub-tropical grasses in WA are:

- Dongara—Kalbarri districts—early to late August
- Perth—Eneabba districts—mid-August to early September
- South coast—early September to early October

This coincides with sufficient soil temperatures for germination and the likelihood of sufficient moisture for good root development before summer.

If soil moisture conditions are unfavourable for sowing, defer pasture establishment until the following year.

5. Set-up the seeder for the best result

Successful establishment of sub-tropical grasses can be achieved with a range of seeding machinery and configurations (tynes, discs) provided the machine:

- forms stable furrows that scalp away non-wetting sand, remove weed seeds and harvests rainfall
- uses press wheels to provide good seed contact with moist soil
- has a wide row spacing (typically 50-60 cm)

Formation of furrows

Sowing into furrows is important to scalp away non-wetting sand, remove weed seeds from near the perennial grass seedlings and harvest rainfall. Furrows capture rainfall and increase seedling survival, particularly in dry springs. They act to turn relatively small rainfall events into useful soil moisture for growth and survival. For example, 3-5 mm of rain can effectively become 10-15 mm for seedlings in the bottom of a furrow.

Furrow depth can be varied according to seasonal conditions. The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting.

Furrow shape should be designed to minimise furrow collapse and sand in-fill, as this causes burial of the seed, which can markedly reduce seedling emergence. Wide furrows with sides that are not too steep are less prone to collapse and sand in-fill.

Seeding without furrows is risky, as it relies on favorable spring-summer rainfall, and is more likely to result in poor or patchy establishment, particularly in years with low spring and summer rainfall.

Press wheels

Press wheels provide good seed contact with soil moisture and lead to more reliable germination. They should press soil around the seed in the furrow bottoms and minimise sand in-fill from the furrow sides. An easy sowing method is to drop seeds into the bottom of furrows and use press wheels to push them below the soil surface. This system positions seeds well for germination, but strong press wheel action is essential to give good seed-soil contact.

The contour of the press wheels should fit within the furrow shape to minimise sand in-fill. The base of the furrow should be wider than the press wheels. Rounded or flat-bottomed wheels tend to produce a better result than angular, narrow wheels, while ”W”-shaped press wheels are the least effective. It is also important to ensure that press wheels are correctly aligned with the furrows and that the arms allow consistent tracking along furrows.

Optimum row spacing

Optimum row spacing appears to be 50-60 cm. This ensures a sufficiently wide inter-row so that any soil movement during sowing from one furrow does not cause sand in-fill into adjacent furrows. It also gives sufficient space for annual pastures to grow between the rows in the cooler months, resulting in a good balance between perennial and annual pasture components.

A wider row spacing can be used in low rainfall areas to reduce competition for soil moisture. Row spacing can also be adjusted to change the balance between winter-spring feed from annual pastures in the inter-row versus potential out-of season production from the perennial grasses.

Close row spacing, such as that achieved with a conventional seeder, can result in greater competition for water, resulting in small, stunted plants and reduced growth following out-of-season rainfall.

Sow different grass species in alternate rows to reduce competition

In mixtures of sub-tropical grasses pasture composition can be dominated by the species with higher seedling vigour. Rhodes grass has the highest seedling vigour of the commonly grown sub-tropical grasses and often out-competes panic grasses and other species when sown together. To overcome this, Rhodes and panic grasses can be sown in alternate rows. Simple dividers can be used in the seed box to allow species to be sown in alternate rows.

6. Sow 2–5 kg/ha of seed

Calibrate the seeder to ensure the correct amount of seed will be sown.

Sow 2-5 kg/ha of seed, depending on seed quality and whether the seed is coated. As a guide, for uncoated seed with 40% germination, 2 kg/ha is sufficient to give a good seedling density. Higher rates should be used for seed of lower germination. Higher rates are also required for coated seeds to sow the same number of seeds per area.

Kikuyu seed normally has high seed quality (> 80% germination) and sowing at 1 kg/ha is usually adequate. However, full groundcover will be achieved more rapidly by sowing at 2 kg/ha.

With light, fluffy seeds like Rhodes grass, it is often necessary to use a carrier if the seed is not coated. Use a low rate of fertiliser (e.g. 20-25 kg/ha) mixed with the seed for better flow through the machinery.

7. Sow at a depth of 5–10 mm

Sub-tropical grasses require shallow should be sown to a depth of 5–10 mm. **Seeds sown too deep will not emerge**. A common method is to drop seeds in the bottom of furrows and press them in with press wheels. When correctly adjusted, a small proportion of sown seeds are usually visible on the soil surface. The bright colour of coated seeds makes checking seeding depth relatively easy. Seeding depth should be checked regularly to ensure that the majority of seeds are being placed 5-10 mm below the surface in the bottom of furrows. This should be done at normal operating speed.

Sowing directly onto the soil surface is unreliable.

8. Don't sow too fast

Tractor speed can impact on establishment success. Driving too fast can cause excessive soil movement, reducing the accuracy of seed placement. Sand in-fill increases and furrows can collapse, resulting in a deeper effective seeding depth and poor resulting establishment. Optimum speed varies with the type of machinery, but from experience speeds of 5-10 km/h generally give a good result.

Some machinery modifications can be made to enable sowing speed to be increased. For example, some producers have added rubber guards to prevent soil from one furrow being thrown into adjacent furrows.

To ensure the majority of seeds are being placed 5-10 mm below the surface sow at normal operating speed and check the results. Check seed placement regularly and make adjustments if needed.

9. Conduct post-seeding checks

There are a number of post-seeding checks that should be conducted to ensure successful perennial grass establishment.

Determine establishment success

Establishment success and pasture composition can be gauged from the time emerging grasses have three leaves. Good establishment should result in continuous lines of perennial grasses in each seeding row across the paddock. Plant density can be determined by counting the number of plants per metre of seeding row. Initial density should be at least 10 plant/m^2 for sowing to be considered successful. Pasture composition can be determined by estimating the relative number of each species.The sown perennial grass seedlings can be identified by comparing with the identification guides in Wintle *et al.* (2010).

Control insect pests

Apply a residual insecticide at sowing or with the last knockdown herbicide to control insects. Monitor the paddock regularly for insect damage, especially over the first 8 - 10 weeks when grass seedlings are emerging and are most vulnerable to insect attack. Control cutworm and webworm caterpillars, redlegged earth mite, Rutherglen bugs and aphids if present.

Control summer growing weeds

Summer growing weeds compete strongly with sub-tropical grass seedlings for soil moisture. Control of these weeds will optimise establishment of sown sub-tropical grasses. Broadleaf weeds such as Afghan and paddy melons, wireweed and fleabane can be readily controlled with a number of broadleaf herbicides, provided they are actively growing.

Couch grass is a highly competitive summer-active perennial grass that will out-compete the more desirable sown perennial grass seedlings during the establishment phase. Care needs to be taken with couch grass, as it often recovers from a knockdown herbicide applied in winter. However, once established, the sown sub-tropical grasses are more productive and compete strongly with couch grass.

Special consideration needs to be given to erosion-prone soils when perennial grass seedling density is low (< 5 plants/m^2). Summer weeds should be retained in such situations to reduce the erosion risk.

Control kangaroos and rabbits

Kangaroos and rabbits can damage or kill young sub-tropical grass plants, so good control is essential. Unrestricted grazing can up-root establishing plants and increase stress over summer, which may reduce their persistence. In particular kangaroos will travel considerable distances to graze sub-tropical grasses, when little other green feed options are available.

10. Defer grazing until grasses are well established

Careful grazing management over the first summer is critical to ensure a strong perennial stand in subsequent years. Sub-tropical grass seedlings have a weak primary root system and as a result are susceptible to up-rooting and grazing damage over the first summer. Uncontrolled grazing during this time will also deplete carbohydrate reserves which can result in plant death.

The timing of the first grazing will vary depending on seasonal conditions and how well the plants have grown. If late spring or summer rainfall has produced vigorous growth, the perennial grasses can be lightly grazed from early January. However, if very little or no summer rainfall has fallen, the first grazing will need to be deferred until after the break of season.

The first grazing should be deferred until the perennial grasses are well established and well anchored.

Before the first grazing assess whether plants are firmly anchored by testing how easily they can be pulled out by hand. What appear to be relatively large plants can have a surprisingly small root system and in that case premature grazing would have a significant negative impact on the stand density with plants being uprooted and killed. Rhodes grass has long runners (stolons) that do not root down in dry soils and is, therefore, particularly susceptible to grazing damage in the first summer (test by pulling on the stolons).

If the plants are well anchored then a light grazing will encourage tillering. Monitor the perennial grasses while stock are in the paddock to ensure they are not being up-rooted or over-grazed.

On-going grazing management will vary with the perennial grass, grazing animal and time of year. Most perennial grass paddocks can be largely set-stocked by cattle during the growing season, but they will require some form of rotational grazing outside of the annual growing season. Kikuyu is highly grazing tolerant and can be set stocked for long periods by sheep or cattle, but leading producers have found large increases in productivity by rotationally grazing kikuyu pastures.

8. Acknowledgements

This project was first conceived by Dr Mike Ewing and Dr Warren Mason and initiated as part of the former CRC for Plant-based Management of Dryland Salinity, which made a direct funding contribution. Funding was also provided by Meat & Livestock Australia, Australian Wool Innovation Ltd and Land and Water Australia. Project management continued in the Future Farm Industries CRC.

We are grateful for the interaction and support of the Evergreen Farming Group. The project team wishes to thank R. Hendrie (‘Flora Downs’, Gillingarra) Q. Fabling (Eneabba) and R. O’Bree (‘Yanget’, Kojarena) and their families for providing land for field trials and assisting with site management and field days. G. Bain and M. Sounness provided valuable input at project planning workshops.

Tim Wiley (formerly DAFWA) was a leading advocate for the use of sub-tropical grasses in the Northern Agricultural Region of WA from the early 1990s until 2009 and provided valuable input at project planning workshops and assisted with identifying field sites.

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Direct seeding of chenopod shrubs for saltland and rangeland environments

Future Farm Industries CRC Technical Bulletin

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Abstract

There are currently two ways of establishing chenopod shrubs: sowing from seed using a niche seeder, or planting nursery-raised seedlings with a tree planter. Planting seedlings is the more reliable method, but is relatively expensive (in excess of $450 per hectare). On the other hand, direct seeding using the specialised "niche seeder" is much less expensive ($100-150 per hectare), but is also less reliable. This project aimed to investigate alternative methods of direct seeding chenopod shrubs for saltland and rangeland areas by developing a greater understanding of their seed biology and agronomic requirements. Our aspiration was that shrubs should be established using more conventional farm machinery.

This bulletin reports on a combination of seed biology and agronomic research to develop reliable, low-cost direct seeding options for chenopod. Experiments into the impact of changing environmental conditions on seeds were studied in the laboratory, and field experiments were conducted testing the applicability of these insights in the field using convential modified farm seeding machinery.

As a result of this work, a successful direct seeding package using farm seeding equipment (modified for wide row spacings and depth control) was developed for *Atriplex nummularia* (old man saltbush), the most widely planted saltbush species across southern Australia. The nine key elements of the package are:

1. Select suitable paddocks for introduction of new shrubs
2. Prepare a weed-free seedbed using two knockdown herbicide applications (4-6 weeks and 1-2 weeks before seeding) and commence control of rabbits and kangaroos
3. Sow the best seed, by ensuring:
    a. Small fruits, with a high proportion of unviable seeds, have been screened off;
    b. Seed is of subspecies *nummularia* (not subsp. *spathulata*);
    c. Fruits have been harvested within the previous 6 months and stored in a cool, dry environment; and
    d. Bracts are retained around the seeds;
4. Sow into moisture in late winter - early spring (depending on district)
    a. If the area to be sown is waterlogged, defer sowing until later in spring
    b. If insufficient soil moisture, defer sowing until the following year
5. Use a sowing rate of ~10 fruits/m (if germination rate is 15%) to provide at least one plant for every 2 m of row; use higher rates for seed of lower germination

6. Set up the seeder to sow into furrows with trailing press wheels;
7. Sow to a depth of 5-10 mm (very critical);
8. Control weeds and pests (insects, kangaroos and rabbits); and
9. Defer grazing until seedlings are well established

This establishment method has also been shown to work for *Rhagodia preissii* (mallee saltbush).

This project was not able to develop reliable direct seeding packages for other *Atriplex* species, including *A. amnicola* and *A. undulata*, and further work is needed to understand the triggers for their germination, before these species can be direct-seeded with conventional machinery. Direct sowing of *M. brevifolia* and *M. pyramidata* appears to be problematic in much of southern Australia, due to their requirement for temperatures >30^{o}C for germination, which do not occur within the normal winter growing season. An exception to this would be areas with more reliable summer rainfall, such as northern New South Wales, where sowing could be deferred until late spring-early summer. An alternative strategy for establishing *M. brevifolia*, is to encourage natural recruitment of seedlings from seed produced on surrounding bushes (if it is already present in the area), or to transplant a low density of nursery-raised seedlings, which could then act as a seed source for natural recruitment (if it is not present).

The first successful direct seeding of *Atriplex nummularia* at Meckering, WA

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1. Background

Much of the Australian landscape is salt affected, with an estimated 5.7 Mha currently salt affected and a further 17 Mha at risk of becoming saline over the next 50 years (NRLWRA, 2001). Saltland pastures, comprised of halophytic chenopod shrubs (family Chenopodiaceae) and other understorey species, have contributed significantly to the productive use of saline land. Three main species of saltbush have been established commercially on saltland: the two Australian natives, *Atriplex nummularia* Lindl. (old man saltbush) and *A. amnicola* Paul G. Wilson (river saltbush), and the Argentinean species, *A. undulata* D. Dietr (wavy leaf saltbush). *Rhagodia preissii* Moq. (Mallee saltbush), another native chenopod shrub, has also been promoted for use on non-saline land, particularly in the northern agricultural region of Western Australia. Other native species, including *A. semibaccata* R.Br. (creeping saltbush), *A. cinerea* Poir. (grey saltbush), *A. bunburyana* F.Meull. (silver saltbush), *Maireana brevifolia* (R.Br.) Paul G.Wilson (small leaf bluebush), *M. pyramidata* (Benth.) Paul G.Wilson (sago bush) and *M. aphylla* R.Br. Paul G.Wilson (cotton bush), are widely naturalised on saltland and methods to either establish them directly or to encourage a greater rate of seedling recruitment, could enhance the productivity of salt affected land. The majority of these species are native to the rangelands and simple methods of establishment could also lead to cost-effective rehabilitation of degraded rangeland areas.

1.1. Establishment techniques

Farmers seeking to establish halophytic shrubs on saltland can either: (a) seed directly using niche seeders, such as the “Mallen” niche seeder (manufactured by W.C. Diamond) and the KimSeed seeder (manufactured by Kimberley Seeds); or (b) they can use commercial tree planters to plant nursery-raised seedlings. The “Mallen” niche seeder was developed by C.V. Malcolm and colleagues in the 1970s (Malcolm and Allen, 1981). It deposits *Atriplex* fruits at 1-3 m intervals (placements) along the centre furrow of a raised M-shaped mound (see Figure 1). The banks are raised to reduce waterlogging and the central furrow acts to capture rainfall and to leach salts from the surface. The fruits are mixed with a vermiculite mulch to reduce salinity and black paint is sometimes added to increase soil temperature. The other niche seeders operate in a similar way.

Direct seeding of saltbush species using niche seeders has had mixed success, and often leads to poor establishment. Firstly, less than 5% of fruits commonly result in successful establishment. Therefore, 50 fruits per placement are recommended, with the aim of obtaining at least one successfully established plant per placement (Barrett-Lennard *et al*. 2003; Moore *et al.* 2006). Secondly, many placements fail to establish any bushes. Vlahos (1997) conducted a survey of saltbush establishment on 63 sites over 22 locations in south-western Australia and found that more than half the sites had less than 25% of placements with successful establishment.

The use of nursery-raised seedlings is generally much more reliable than niche seeding (Barrett-Lennard *et al*. 1991). However, direct seeding (~$100–150/ha) is far cheaper than the planting of nursery-raised seedlings (more than $450/ha). Farmers, therefore, face a trade off between risk and price: the establishment technique that is most reliable is too expensive to implement; the accessibly priced technique is too risky. Furthermore, the cost of sowing these species for rehabilitation of large pastoral zone areas is currently prohibitive and too risky. If prices for *demonstrably reliable* saltland and rangeland pasture establishment can be brought down to ~$120/ha, there would be substantial farmer and grazier adoption. Furthermore, there is a high chance that the developed techniques would be appropriate to the adoption of direct seeding for applications in the rangeland and arid areas of the wheatbelt.

Figure 1. Direct seeding with the "Mallen" niche seeder.

There are two approaches to developing direct seeding techniques for more reliable chenopod shrub establishment. One is to better engineer the sowing niche. Maximum growth of shrubs is possible with stands of ~1,000 stems per hectare (Malcolm *et al*. 1988; Barrett-Lennard 1993). Thus, an outlay of \$100–\$120 per ha for establishment equates to a cost of 10–12 cents per seed placement. This allows for the possibility of relatively expensive micro-engineering options to help overcome a number of stresses affecting establishment including salinity, waterlogging, inundation, drought, weeds, insects, grazing, and low temperatures or frost (Malcolm, 1972; Jennings *et al*. 1993; Barrett-Lennard *et al*. 2003). The alternative viewpoint is to develop direct seeding methods in which farmers can use their own seeding equipment. This project elected to follow this approach, with the main target area being those more favourable to chenopod growth - mildly to moderately saline areas, not subjected to prolonged waterlogging. These areas are also likely to support growth of grass and leguminous understorey species that can markedly increase livestock production (Barrett-Lennard *et al*. 2005; Norman *et al*. 2010).

1.2. Seed biology

Relatively little is known about the seed germination requirements of many of the main chenopod shrub species of interest. A greater understanding of their seed biology and the environmental factors that trigger germination is needed, before more reliable establishment methods can be developed. Such issues include physical or embryo dormancy, the role of bracteoles enclosing the seed, and optimal temperature and moisture conditions for germination.

Given the harsh micro-environment in which *Atriplex* and other halophytic species grow, often characterised by high salinity and drought, the development of seed treatments that maximise the potential of the seed to germinate and emerge rapidly could significantly enhance establishment success (Powell 1998). This includes priming (pre-germinating) seeds with water or germination stimulants. This technique has been successfully used for a range of species with plant signalling chemicals including karrikinolide (KAR1, the active ingredient in smoke, formerly known as butenolide) (Flematti *et al.,* 2004), gibberellic acid ($GA_{3,}$) benzoic acid (Senaratna *et al.*, 2003), salicylic acid (Senaratna *et al*., 2000) and cytokinins.

In a pilot project, Kings Park and Botanical Garden and the Department of Agriculture and Food Western Australia (DAFWA) identified several possible dormancy alleviation and germination stimulation strategies for old man saltbush, river saltbush and wavy leaf saltbush, each of which differs in germination requirements (Stevens *et al.* 2006). For *A. amnicola*, the presence of light and the substitution of light by 1000 ppm of GA_3 improved germination under laboratory conditions and resulted in a four-fold increase in seedling emergence in the field. For *A. undulata*, removing bracteoles from the fruit increased germination under by 15% under laboratory conditions and gave a 1.5-fold improvement in seedling field emergence. On the other hand, bracteole removal and light had small positive effects on germination of *A. nummularia* under laboratory conditions, but this did not translate into improved emergence of seedlings in the glasshouse or field. The effects of seed priming on seedlings varied with species. Priming with water significantly increased emergence percentage of river saltbush, but had no effect on old man or wavy leaf saltbushes. Salicylic acid and kinetin improved the rate of emergence of all three species at various levels of salinity, while GA_3 also improved germination of wavy leaf saltbush.

This bulletin reports on a combination of seed biology and agronomic research to develop reliable, low-cost direct seeding options for chenopods. It examines the potential of seed treatments identified by Stevens *et al.* (2006) for use in the field and the development of new seeding equipment solutions. In particular, the use of conventional farm machinery for sowing is examined, to encourage farmers to sow their own seed.

2. Project details

A four-year project, titled “Reliable establishment of non-traditional perennial pasture species”, commenced on 1 July 2006 as part of the former Cooperative Research Centre for Plant-based Management of Dryland Salinity (Salinity CRC), with industry funding from Meat & Livestock Australia, Australian Wool Innovation and the former Land and Water Australia. Management of the project was continued by the Future Farm Industries Cooperative Research Centre (FFI CRC), upon its inception in July 2007. The project consisted of four sub-projects: (i) “Establishment of saltland and rangeland species”; (ii) “Establishment of exotic warm-season grasses and legumes”; (iii) “Establishment of native grasses and legumes”; and (iv) “Recruitment of native grasses in native pastures”. This bulletin focuses on results from the sub-project “Establishment of saltland and rangeland species”.

The saltland pastures component of the project consisted of interlinked laboratory and field components. Kings Park conducted laboratory and glasshouse studies to gain an understanding of the seed biology and germination requirements of the species and also examined a range of potential germination enhancing seed treatments. DAFWA used this information to develop agronomic and seeding machinery solutions to develop agronomic packages for establishment.

2.1. Species examined

Ten species (see Table 1) were examined during the project; their fruits and seeds are shown in Figure 2. However, most work was conducted with the main commercially utilised species, *Atriplex nummularia*, *A. amnicola, A. undulata* and

Maireana brevifolia. The greatest success in developing a reliable establishment package was acheved with *A. nummularia*; this will be focussed on here in greatest detail.

There are two recognised subspecies of *Atriplex nummularia* Lindl. - *Atriplex nummularia* Lindl. subsp. *nummularia* and *Atriplex nummularia* subsp. *spathulata* Aellen (Anon. 2011). Subspecies *spathulata* can be distinguished from subsp. *nummularia* by having smaller fruits and much shorter petioles. The two species are shown in Figure 3. Subspecies *nummularia* generally has higher nutritive value and palatability characteristics than subspecies *spathulata* (H. Norman, pers. comm.). Subspecies *nummularia* was, therefore, used in the laboratory and most of the field studies.

Table 1. Species examined in this Bulletin.

Scientific name	Common name
Atriplex nummularia Lindl.	Old man saltbush
A.amnicola Paul G.Wilson	River saltbush
A. undulata D. Dietr	Wavy leaf saltbush
A. semibaccata R.Br.	Creeping saltbush
A. bunburyana F.Meull.	Silver saltbush
A. cinerea Poir.	Grey saltbush
Maireana brevifolia (R.Br.) Paul G.Wilson	Small leaf bluebush
M. pyramidata (Benth.) Paul G.Wilson	Sago bush
M. aphylla (R.Br.) Paul G.Wilson	Cotton bush
Rhagodia preissii Moq.	Mallee saltbush

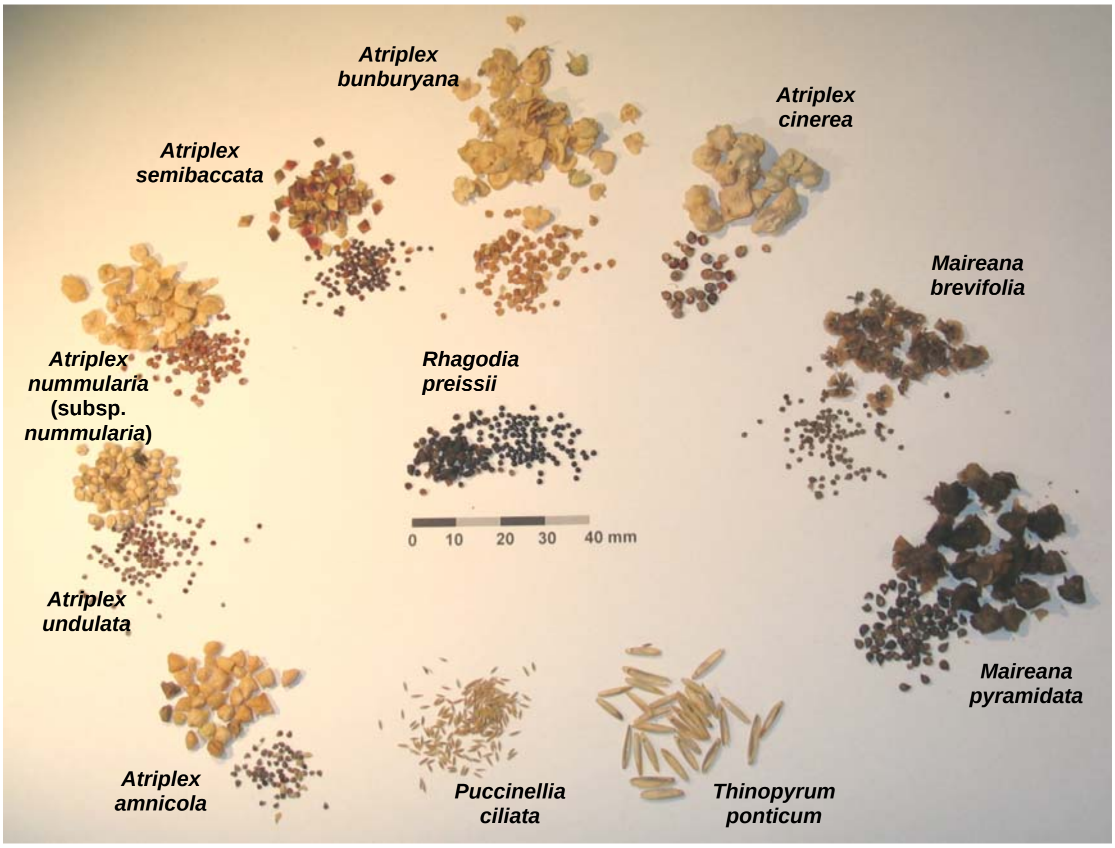


Figure 2. Fruits and seed of *Atriplex amnicola*, *A. undulata*, *A. nummularia*, *A. semibaccata*, *A. bunburyana*, *A. cinerea*, *Maireana brevifolia*, *M. pyramidata* and *Rhagodia preisii*. Two commonly sown saltland perennial grass species, *Puccinellia ciliata* and *Thinopyrum ponticum*, are also shown.

Figure 3. Fruits and leaves of subsp. *nummularia* (left hand side) and subsp. *spathulata* (right hand side) of *Atriplex nummularia*.

3. Seed biology studies

3.1. Seed quality

Seed batches can vary widely in quality. Data from Vlahos *et al*. (1991) showed that seed batches of *A. amnicola* often had less than 25% germinable seed, with less than 10% of samples having more than 50% germination. Seed quality is affected by seed age, purity, dormancy and the proportion of weed seeds. Poor seed quality results in low germinability and is a major limitation to successful establishment from direct seeding. “Seed purity” is defined as the percentage of viable seed by weight in a seed batch. High quality seed batches have high levels of seed purity. However, if there is a high proportion of dormant seed, the seed batch may have high seed purity, but low germinability. Once this dormancy is overcome, the seed is then capable of high germinability.

Three factors affect seed quality: (i) the amount of seed fill in the fruits at harvest; (ii) the amount of inert harvest debris, empty and damaged seeds in the seed batch; and (iii) the duration and conditions of storage. The amount of seed fill at harvest is affected by plant maternal environment, particularly the extent of fertilisation and embryo abortion (Barrett-Lennard *et al*. 2003). Some of the important chenopods for saltland are dioecious, with male and female flowers occurring on separate bushes, while others are monoecious.

Fertilisation occurs when wind-blown pollen reaches female flowers. If pollination does not occur, fruits develop, but embryos do not. Saltbush fruits ripen over a period of 6-7 months. A proportion of embryos do not develop, with the proportion being higher if plants are under stress. (Barrett-Lennard *et al*. 2003). For example, Strawbridge (1995) found in *A. amnicola* across three different sites that 80-90% of developing fruits contained embryos in early seed development (May-June), but when fruits were ripe in November-December, only 25-45% of fruits contained embryos.

Harvest timing is also critical and is a compromise between maximum seed set and the risk of seed shedding. Seed batches can contain empty florets, immature seed, dormant seed and dead seed in addition to viable seed; these amounts will form a higher proportion of the seed sample if the seed cleaning process is inefficient. Sufficient time is needed post-harvest to overcome any after-ripening seed dormancy issues. This is influenced by seed water content and ambient temperature, while extended lengths of storage under sub-optimal conditions can desiccate and decay seed embryonic tissue.

In general saltbush seed germination decreases over time (Barrett-Lennard *et al*. 2003). Therefore, once any seed dormancy issues have been overcome, the freshest seed possible should be used. Storage affects the longevity of seed in varying ways. Beadle (1952) showed that *A. nummularia* seeds were most germinable within four years of age, but eight years after harvest germinability had declined from 92% to 10%, while for *A. vesicaria*, germinability declined to 0% after five years.

In the present study, some experiments were conducted to provide further understanding and quantification of factors limiting seed quality of priority species.

Materials and methods

Seed quality was determined in ten seed batches of 8 chenopod species acquired from commercial companies and from DAFWA seed increase plots. The commercial seed batches represented samples sold over the counter to farmers.

The process for determining seed quality was as follows. Debris was first separated from fruits by hand. Filled seeds (those with well developed embryos) within the fruits were identified by counting with a Faxitron MX-20 x-ray machine to assess the result of the cleaning process (see Figure 4). Seeds (4 replicates of 25) were then incubated in moistened Petri dishes over a 14 day period at 16^{o}C, after which the seed purity of the batch was calculated. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

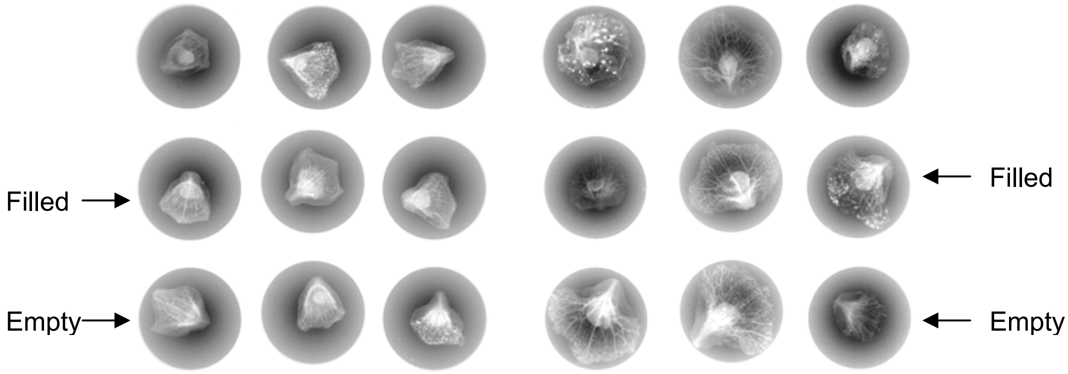


Figure 4. X-ray plates taken with a Faxitron MX-20 showing empty and filled fruits (containing well developed embryos) in the bracts of *Atriplex nummularia* (left) and *A. amnicola* (right).

Results and discussion

Table 2 summarises the seed quality results. Seed fill percentage of several batches was low, including *A. undulata* (34%), *A. nummularia* (54%) and *A. amnicola* (57% and 66%), while the three *Maireana* and *A. bunburyana* batches all had > 85% seed fill. Germination of the filled fruits was lowest for *M. brevifolia* (36% and 43%) and highest for M. pyaimidata (97%). The two *A. amnicola* batches ranged from 55% - 82%, indicating differences within the species. Percentage seed purity was lowest for the *A. undulata* batch (57%), while the *A. amnicola* and *A. nummularia* batches had 75% seed purity.

The x-ray machine was much more successful in identifying fruits filled with well developed embryos than visual assessments, as empty seeds or seeds encased in bracts or florets often looked the same as filled fruits.

Observations with a range of species suggest that morphological seed properties favouring separation from harvest debris and the threshing ability of seed cleaning equipment play an important role in the viability of cleaned seed. For example, in contrast to the chenopods, legume seed batches tend to have a much higher level of purity, as they are easily separated from debris, due to their heavier seed weight and uniform seed size. The light fruits and encasing of the seed within bracteoles makes chenopod seeds more difficult to separate from debris during threshing.

These factors may contribute to the poor seed purity with many chenopod seed batches.

Table 2. Summary of seed quality attributes of commercial seed batches of 8 chenopod shrub species. Tests were undertaken after a minimum drying period of 14 days at 16^{o}C and 25% relative humidity.

Species	% filled fruits[1]	% germination of filled fruits[2]	% seed purity by weight of viable seeds[3]
Atriplex amnicola (a)	57	55	70
A. amnicola (b)	66	82	77
A. undulata	34	87	57
A. nummularia	54	93	71
A. semibaccata	70	67	79
A. bunburyana	89	70	90
A. cinerea	77	89	76
Maireana brevifolia (a)	86	36	94
M. brevifolia (b)	91	43	95
M. pyramidata	86	97	90

[1]Percentage of fruits filled with seed as determined by x-ray analysis

[2]Percent germination after 14 days of fruits screened for >95% filled seed

[3]Seed purity of unscreened sample, denoted by percentage by weight of viable seeds

3.2. Seed dormancy

Following harvest, many species display a period of post-harvest seed dormancy, where seeds are viable but are not able to germinate. High levels of seed dormancy at sowing present a problem for two reasons. Firstly, establishment is likely to be much lower than expected given the seeding rate. Secondly, seeds that later come out of dormancy and germinate on summer-autumn rain are less likely to successfully establish.

Many chenopods exhibit physiological dormancy that is usually relieved over a period of time, whereby seeds undergo biochemical and physiological changes enabling seeds to eventually germinate. Seeds in this state are usually referred to as being in a state of 'primary innate dormancy'. As seeds fulfil their after-ripening requirement, they then enter conditional dormancy, during which they tend to germinate over a narrow range of environmental conditions. During the progression of dormancy loss, the range of conditions over which the seed will germinate gradually widens, until it is able to germinate over the full range of conditions as dictated by the genotype.

The rate at which dormancy loss occurs has been linked to temperature and moisture conditions experienced by the seed. Non-dormant seeds of some species may re-enter dormancy if environmental conditions remain unfavourable for germination. Such seeds become conditionally dormant then eventually completely dormant. There is little or no information in the literature pertaining to after-ripening and patterns of dormancy for most project species, particularly those of highest priority. An understanding of these characteristics is important for the seed industry, particularly where storage and germinability of product are concerned.

Research to date indicates that secondary dormancy exists for *M. brevifolia* and *R. preissii*. Seed stocks of these species have been relieved of dormancy through the use of plant signalling agents (see chemical seed enhancement section).

3.3. Temperature requirements and optimum sowing time

The interactions of temperature, light and moisture play a critical role in regulating seed germination in the field. A series of experiments was conducted to determine the optimum temperature and light treatments on germination. This information provides a basis to estimate best seasonal "sowing windows" in the field to maximise germination and emergence, particularly for lesser-known species. It also forms the basis for comparing the effects that physical and chemical germination enhancement treatments might have in modifying these "sowing windows."

Materials and methods

Seeds (4 replicates of 25) were incubated in Petri dishes with constant temperatures (light/dark) in the range between 5°C and 40°C or with alternating temperatures (12/12hr light/dark) of 18/7°C, 26/13°C or 33/18°C. These temperatures represent typical late winter, early to mid spring and late spring to early summer field temperatures, respectively, in southern Australia. Light/dark and dark/dark regimes were only compared at 20°C.

Results and discussion

The optimum temperature regimes for germination are presented in Table 3. Also shown are proposed "sowing windows" for each species, based on comparing optimum temperature regimes with typical field temperatures in the central wheatbelt of Western Australia (WA). These "sowing windows" are a guide only and need to be modified for cooler and warmer regions of southern Australia. Sowing times also need to be considered in the context of surviving summer drought and the ability of seedlings to compete with weeds and insects. So, while a species may be suited for germination in late winter to spring, growth and biomass production may not be sufficient to enable seedlings to survive summer drought; such species could be sown in autumn to give plants extra time to establish.

Atriplex nummularia, *A. undulata* and *A. semibaccata* have germination temperature regimes best suited to late winter to early spring sowing, while *A. amnicola*, *A. bunburyana* and *A. cinerea* appear better suited to spring sowings. *A. cinerea* has equally good germination across alternating and constant temperatures, suggesting this species is well suited to the northern agricultural regions of WA and New South Wales (NSW), with the possibility of expansion into the northern rangelands. Sufficient soil moisture for germination and early growth is also critical in, addition to optimum temperatures; late sowing is risky in southern Australia, with establishment success being dependant on good root development prior to onset of the summer drought.

M. brevifolia and *M. pyramidata* have higher temperature requirements for germination and appear better suited to germination in late spring and summer. This clearly presents difficulties for direct seeding in much of southern Australia, where summer rainfall is sporadic and unreliable. However, these species could be established more reliably in areas with a higher reliability of summer rainfall, such as northern NSW and Queensland.

Table 3. Constant temperature ranges and alternating temperature gradients for maximum germination percentage (%G) after 14 days for 8 chenopod shrub species. The potential sowing window in the central wheatbelt of Western Australia, corresponding to these temperatures, is also shown (in yellow).

Species	Optimum temperatures (^{o}C)		Potential sowing window[1]
	Constant	Alternating	
Atriplex amnicola	15-25	18/7-26/13	J F M A M J J A S O N D
A. undulata	10-20	26/13-33/18	J F M A M J J A S O N D
A. nummularia	10-20	18/7-26/13	J F M A M J J A S O N D
A. semibaccata	15	18/7-26/13	J F M A M J J A S O N D
A. bunburyana	15-25	18/7	J F M A M J J A S O N D
A. cinerea	20-30	26/13-33/18	J F M A M J J A S O N D
Maireana brevifolia	25-30	26/13-33/18	J F M A M J J A S O N D
M. pyramidata	25-30	26/13-33/18	J F M A M J J A S O N D

[1]Late sowing risky in southern Australia and dependant on plant available water

3.4. Genotypic differences in A. nummularia *for ability to establish from direct seeding*

Work conducted by Clive Malcolm in the 1980s demonstrated that some *A. amnicola* bushes had more seedling recruitment surrounding the mother plant than others (Moore *et al.* 2006). He also showed that seed derived from such plants had a greater ability to establish from seed and that this was a heritable effect (Malcolm *et al*. 2003).

Similar differences in the amount of seedling recruitment were observed between bushes of *A. nummularia*. This led to the hypothesis that genotypic differences occur within *A. nummularia* for ability to establish from direct seeding and that such plants are derived from bushes that recruit seedlings more readily.

Materials and methods

Seeds of *A. nummularia* ssp. *nummularia* were collected from the property of Michael Lloyd at Pingaring, Western Australia. The saltbush stand had been sown 16 years previously. Seeds were collected from three types of bush: (A) bushes from the original sowing with no surrounding seedling recruits (Mature non-recruiter); (B) bushes from the original sowing with a high density of seedling recruits surrounding them (mature recruiter); and (C) young plants in close proximity to mature recruiters, which were presumably derived from type B bushes (Immature recruiters). This is shown diagrammatically in Figure 5. Seeds were collected on three plants of each type on 28 February 2009.

Two glasshouse experiments were conducted to determine whether seeds from bushes with high recruitment levels established more readily when sown into soil. These were conducted to confirm that emergence differences were seed related and not confounded by preferential predation and grazing or different soil types at the collection site.

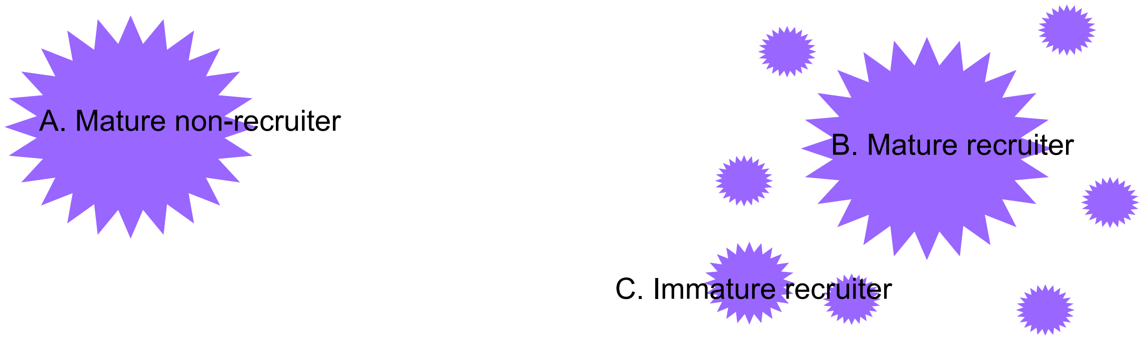


Figure 5. Diagrammatic representation of the three types of *Atriplex nummularia* bushes from which seed was collected.

In both experiments fruits were first checked for seed fill using the Faxitron MX-20 x-ray machine and any unfilled seeds were removed from the sample by hand. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for analyses.

Experiment 1 aimed to examine emergence of seedlings from seeds collected from the three different types (A, B and C) of bush. Three bushes of each type were tested (9 treatments in total). Twenty-five bracted seeds from each bush were planted to a depth of 5 mm in free-draining 150 mm diameter pots filled with non-saline sand and patted down firmly. The experiment contained 4 replicates. Pots were watered daily. Seedling emergence was counted every 7 days for 42 days. Counts were adjusted for viability.

Experiment 2 examined the results from the first experiment more closely. Two contrasting types were used, an Emergent type (designated Bush I) and a Semi-emergent type (designated Bush G) (Table 4). For this experiment only confirmed viable seeds were used. Four replicates of 25 bracted seeds were sown into two soil types, sand and a loam. Seeds were sown in 150 mm diameter free-draining plastic pots to a depth of 5-7 mm and patted down firmly. Pots were watered daily. Emergence was scored every 7 days over a 21 day period.

Results and discussion

For Experiment 1, germination percentage after 35 days is shown in Table 4 (also Figure 6). Bushes were categorised as being “Emergent” if their germination of viable seeds was >75%, “Semi-emergent” if their germination was 25-74% of viable seeds and “Non-emergent” if their germination was <25% of viable seeds.

The results indicate that the recruiting pattern of genotypes of *A. nummularia* do not necessarily indicate high germinability when sown at a depth of 5 mm. Some individual genotypes, such as those from Bush I (an Emergent type), had high viability and good germinability, while others, such as those from Bushes A and D (Non-emergent types), had high viability and low germinability. Notably, the ability to recruit seedlings had no influence on their ability to emerge from sowing. For example, Bush B (an Emergent type) was classed as a mature non-recruiter but had 100% germination. Furthermore, when germination percentages were averaged within bush types, the mature non-recruiter, mature recruiter, and immature recruiter types had germination values of 69%, 56% and 67%, respectively (data not shown).

Table 4. Percentage of viable seed and germination percentage with standard errors 35 days after sowing of seedlings derived from different *Atriplex nummularia* bushes, when sown at 5 mm depth (mean of 4 replicates).

Bush No	Bush type	% viability	% germination	Emergence type
A	Mature non-recruiter	44%	15 ± 2.5	Non-emergent
B	Mature non-recruiter	52%	100 ± 1.9	Emergent
C	Mature non-recruiter	72%	86 ± 1.2	Emergent
D	Mature recruiter	28%	13 ± 2.0	Non-emergent
E	Mature recruiter	48%	42 ± 2.0	Semi-emergent
F	Mature recruiter	56%	87 ± 9.0	Emergent
G	Immature recruiter	72%	55 ± 4.1	Semi-emergent
H	Immature recruiter	52%	79 ± 1.9	Emergent
I	Immature recruiter	72%	100 ± 4.3	Emergent

Figure 6. Glasshouse pot experiment showing differences in emergence of *Atriplex nummularia* seedlings derived from different bushes from Pingaring, Western Australia

In Experiment 2 the Emergent type (Bush I) had markedly higher emergence 21 days after sowing than the Semi-emergent type (Bush G) in both soils (Figure 7), confirming the results of Experiment 1. There was a soil type effect for the Emergent type, with an emergence rate in the loamy soil of almost 80%, compared to 38% in sandy soil. This difference was not seen in the Non-emergent type.

These results show (unfortunately) that the likely germination success of seed lots cannot be predicted based on the observations of plant recruitment around mother plants in the field. Rather, actual measurements of establishment from individual seed lots are required. However, multiple copies of individual parent plants ("mothers" and "fathers") can be made from cuttings (Barrett-Lennard *et al*. 2003). There may be opportunities for commercial seed merchants (working with researchers) to develop their own propriety saltbush lines, selected for high establishment by: (a) identifying combinations of male and female plants that produce germinable seed; (b) cloning the two parent plants involved (the female will be known, the male will be from a nearby pollen source); and (c) establishing seed production nurseries using this material.

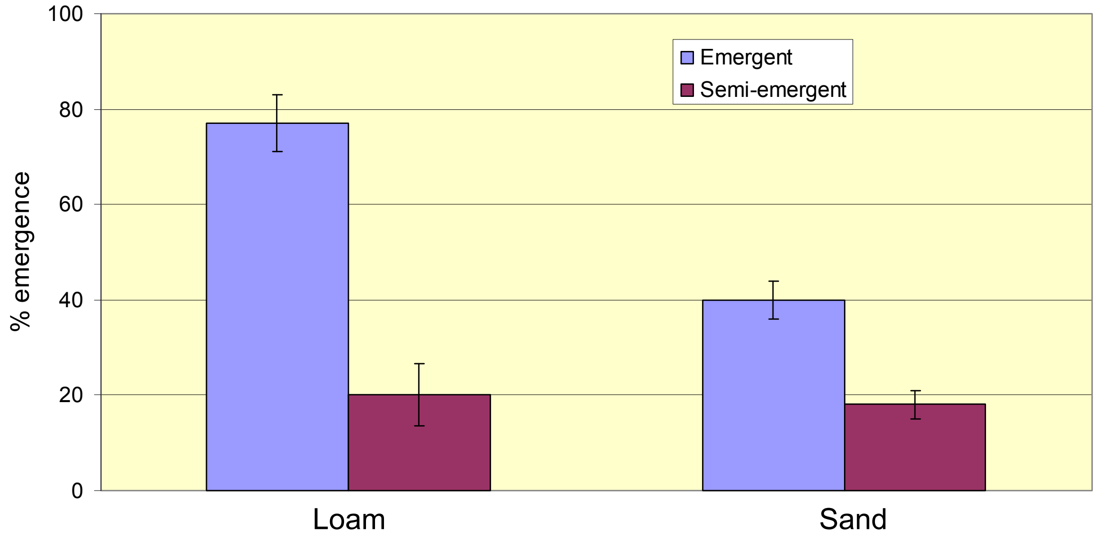


Figure 7. Percent emergence after 21 days in either a sand or loamy soil of an Emergent and Semi-emergent genotype of *A. nummularia* grown in the glasshouse.

3.5. Germination response to temperature in types with different abilities to emerge

Differences in emergence responses between seeds from *A. nummularia* Bush I and Bush G could be explained by differences in their temperature requirements for germination. Therefore, the relationship between temperature and germination was examined in both genotypes.

Materials and methods

Four replicates of 25 naked seeds of each genotype were plated into 90 mm vented Petri dishes containing 0.6% water agar solution, impregnated with 0.1% plant preservation mixture. Petri plates were then sealed with plastic wrap and placed in alternating 12/12 hour temperature cabinets set to 20/10°C, 26/13°C and 35/20°C under a diurnal light/dark regime. Germination was scored every second day over a 14 day incubation period.

Results and discussion

There were no major differences in germination percentage between the Emergent and Semi-emergent *A. nummularia* genotypes in the 20/10°C and 26/13°C temperature regimes (Figure 8). However, a marked difference was found at 35/20°C, with the Semi-emergent type having a much lower germination percentage than the Emergent type. At this temperature regime, germination percentage of the Emergent type was also significantly less that at 20/10°C. This result suggests that emergent saltbushes may be better at withstanding the high temperatures that can occur on some days in spring.

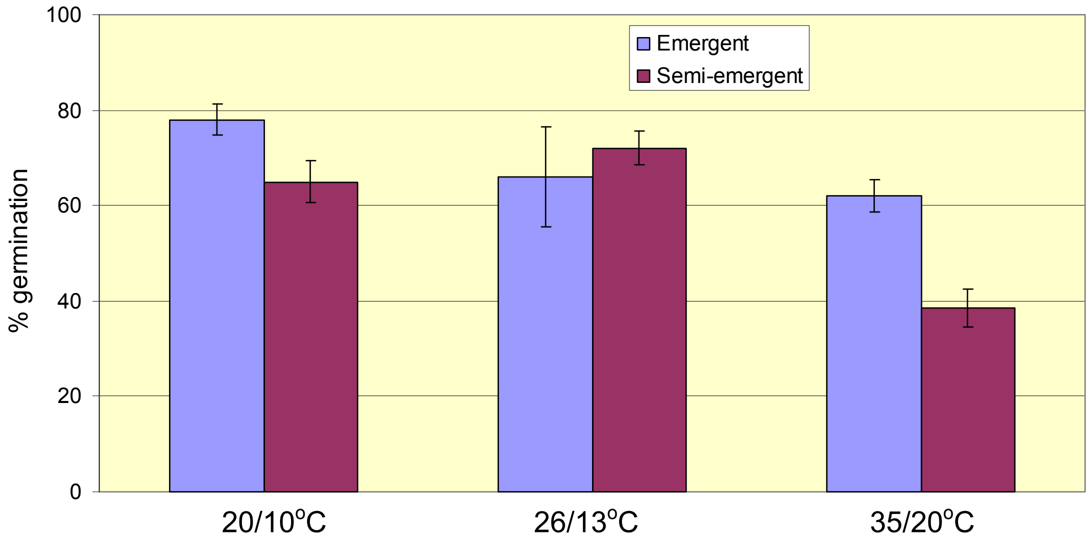


Figure 8. Percent germination after 14 days of Emergent and Semi-emergent types of *A. nummularia* in the laboratory under three different alternating temperature regimes

3.6. The role of bracteoles in germination

Seeds of most Chenopod species are surrounded by lignified bracts, which can vary from being small and light in *Atriplex nummularia* to heavily lignified (woody) in *Maireana pyramidata* (see Figure 1). It is believed that these bracteoles play an ecological role by regulating the timing of germination and aiding in seed dispersal (Ungar and Khan 2001). Laboratory studies by Stevens *et al.* (2006) found bracteoles retard germination of *A. nummularia*, *A. undulata* and *A. amnicola* seed. They also found that debracted seed of *A. amnicola* germinated and survived better under field conditions, whereas bracted seed of *A. nummularia* performed better.

Two genotypes of *A. nummularia*, obtained by Ron Yates from the property of Michael Lloyd at Pingaring, Western Australia were examined. One of these (an Emergent type) was obtained from Bush H, that was found to have high emergence rates in the glasshouse when sown at 5 mm depth. The other (a Non-emergent type) was from Bush A, which was found to have low emergence rates in the glasshouse. This provided a unique opportunity to compare seed and bract properties between plants from the same environment to gain an understanding of the factors responsible for high emergence and establishment in bracted and naked seed.

Laboratory experiments were conducted to examine germination following bract removal to repeat the results of Stevens *et al.* (2006) in other chenopod species.

Materials and methods

Bracts from most Chenopod species were removed using a Kimseed scarifier set at 1.6 - 2.2 mm plate width. This method was effective at removing bracts without damaging the seed. *Maireana brevifolia* bracts, however, were removed by hand rubbing on a rubber mat, due to fragility of the seeds. De-bracted seeds were separated from debris using sieves and a vacuum aspirator.

Bracted seeds with high viability were selected for experiments using a Faxitron MX-20 x-ray machine to ensure seeds within bracts had fully developed embryos. Treatments one and two consisted of bracted and de-bracted seeds plated onto Petri dishes lined with glass filter papers. The germination medium for treatment three used bracts from individual species ground to <500 μm, autoclaved and spread evenly to a 0.5 mm depth. This treatment was designed to investigate the presence of germination-inhibiting allelopathic compounds in the bract material. All treatments were conducted under an 18/7°C 12/12hr regime temperature under dark conditions.

Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index was calculated using equations 1 and 2.

Final germination % at $t = [\Sigma G_t / r] \times 100$ (1)

Where: t = incubation period (days);

G = total germinated seeds across treatment Petri dishes;

r = number of replicates

Germination rate index (GRI) = $\sum[(D_i - D_{i-1})/_i]$, (2)

Where: I = is the germination count day;

D_i = the percentage of seeds germinated at time "i";

D_{i-1} is the percentage of seeds adjudged germinated the previous count day (from Maguire, 1962).

Results and discussion

Table 5. Effect of de-bracting chenopod seeds on percentage germination and germination rate index (GRI) after 14 days in Petri dishes. All de-bracted seed treatments were significantly different (P<0.01) to controls.

Species	Bracted seed (control)		De-bracted seed (additional % compared to control)	
	% germination	GRI (% germination/day)	% germination	GRI (% germination/day)
Atriplex amnicola	24	3	58	17
A. nummularia	62	9	35	36
A. undulata	56	6	29	15
A. semibaccata	49	9	18	4
A. bunburyana	72	14	0	12
A. cinerea	6	0.5	90	26
Maireana brevifolia	17	7	19	4
M. pyramidata	0	0	97	40

For most Chenopod species, de-bracted seed substantially enhanced both germination percentage and germination rate under laboratory conditions (Table 5). De-bracting appeared to be a necessity for reliable germination of *Maireana* *pyramidata* and *Atriplex cinerea*, but had only a small germination enhancing effect on A. *bunburyana*. The results for *A. amnicola*, *A. undulata* and *A. nummularia*, were similar to the results of Stevens *et al.* (2006).

3.7. Emergence of bracted and de-bracted seed of Emergent and Non-emergent A. nummularia *types*

Field evidence in Section 4.5 suggests naked (debracted) *Atriplex* seeds tend to establish better than bracted seed in lower areas of the landscape, where there is greater soil moisture. This suggests that bracteoles may regulate seed germination through either physical properties, such as their ability to hold water, regulate water uptake or buffer moisture loss during drying, or osmotic adjustment, due to the presence of salts. Underpinning germination is a requirement for seeds to reach a critical seed moisture content, in order for germination to proceed. Factors responsible for a seed within a bracteole having a reduced capacity to reach the critical seed water content could be: (i) bracteole structure impeding the flow of moisture; (ii) seed testa properties presenting a moisture barrier; or (iii) potential osmolytic effects from salts that may be present in the bracteoles.

3.7.1. Germination under moist and dry conditions

Materials and methods

Seeds of the Emergent and Non-emergent *A. nummularia* types were dried at 16^{o}C, 25% relative humidity for seven days and then separated from inert material with a vacuum aspirator. Bracted seed was x-rayed using the Faxitron MX-20 x-ray machine and any unfilled seeds were removed from the sample by hand. Following germination testing, any ungerminated seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for analyses. For each genotype, one batch of seeds (naked seeds) was de-bracted gently using a serradella dehuller and separated from inert material using a vacuum aspirator. The other batch had intact bracts. The germinability of *A nummularia* seed sources was compared with a single source of *A. amnicola* seed (naked or bracted).

Two groups of 48 pots (150 mm diameter) were set up in an open area at Kings Park between April 23 and May 4, when average temperatures were 27.6/13.5°C (Bureau of Meteorology 2009). Half the pots were filled with loam and the other half filled with sand. In each pot either 30 naked or bracted seeds of the Emergent and Non-emergent *A. nummularia* types or the naked or bracted *A. amnicola* were planted to a depth of 5 mm. Four replicates of each species per moisture treatment were used and these were randomly positioned within the treatment area. Pots allocated to the “wet” treatment were watered daily while pots allocated to the “dry” treatment were hand watered once every five days. A moisture probe (MPM-160-B, ITC international) was used to measure pot soil moisture contents. Five random pots containing sand and eight of loam from wet and dry irrigation treatments were selected weekly and measured at 10:00 am. The number of emerged seedlings was scored twice weekly for 21 days, with a further measurement after 30 days.

A laboratory control was set up using four replicates of Petri dishes for bracted and naked seeds of both genotypes of *A. nummularia* and for *A. amnicola*. For each replicate, either 25 bleached naked or bracted seeds were placed on filter paper (Whatmans No. 1) with 8 mm of tissue culture water. The naked and bracted seeds were bleached for 7 and 10 minutes, respectively, before being plated. The plates were incubated at 26/13°C on a diurnal light/dark regime (Contherm 6000 CP), and the number of seedlings which germinated was recorded every two days until germination ceased. Germination was defined as emergence of a 1 mm root radicle, provided that growth continued.

Data were analysed by one-way ANOVAs, unpaired t-tests and the Tukey test at a significance level of α = 0.05 using Microsoft Excel 2007 and GenStat version 11. Percentages and averages are presented with standard errors.

Results

On average the water content of the dry pots was 14.0 ± 0.72%, compared to the wet pots with 18.9 ± 0.39%. The wet pots filled with loam had a significantly higher water content (19.7 ± 0.68%) than the pots filled with sand (16.3 ± 0.58%) (t = 0.042).

Figures 9 and 10 show that bracted seeds of *A. amnicola* had a lower emergence rate than naked seeds in both soil types and moisture treatments. However, emergence rate was higher in the loam than the sand for both the bracted (t < 0.01) and naked seeds (t < 0.01). Within the loam, there was no significant difference between the dry and moist treatments for either bracted (t = 0.38) or naked seeds (t = 0.07), nor was there a significant difference between the moist and wet treatments for bracted (t = 0.18) and naked seeds (t = 0.754) in the sand.

Overall, bracted seeds of *A. nummularia* had a lower emergence rate than naked seeds (Figures 9 and 10). The exception to this was bracted seeds of the Non-emergent type, which had a 35% greater emergence rate than naked seeds in the moist treatment in loamy soil (Figure 10). Furthermore, bracted seeds of the Emergent type had a higher emergence rate in the sand than the loam (t <0.001). There was no statistical difference between the emergence of naked seeds of the Emergent type and either bracted or naked seeds of the Non-emergent type in the two soils. There were also no significant differences between the emergence of naked or bracted seeds in the dry or moist treatments, apart from: (i) bracted seeds of the Non-emergent type, which had a higher emergence rate in the moist loam than the dry loam (t = 0.039); and (ii) the dry and moist sand treatments for both the bracted (t <0.001) and naked (t =0.02) seeds of the Non-emergent type.

Naked seeds of *A. amnicola* and both *A. nummularia* types also had a greater germination rate than bracted seeds in the laboratory control (Figure 11). The bracted seeds of *A. amnicola* had the highest germination rate of all species.

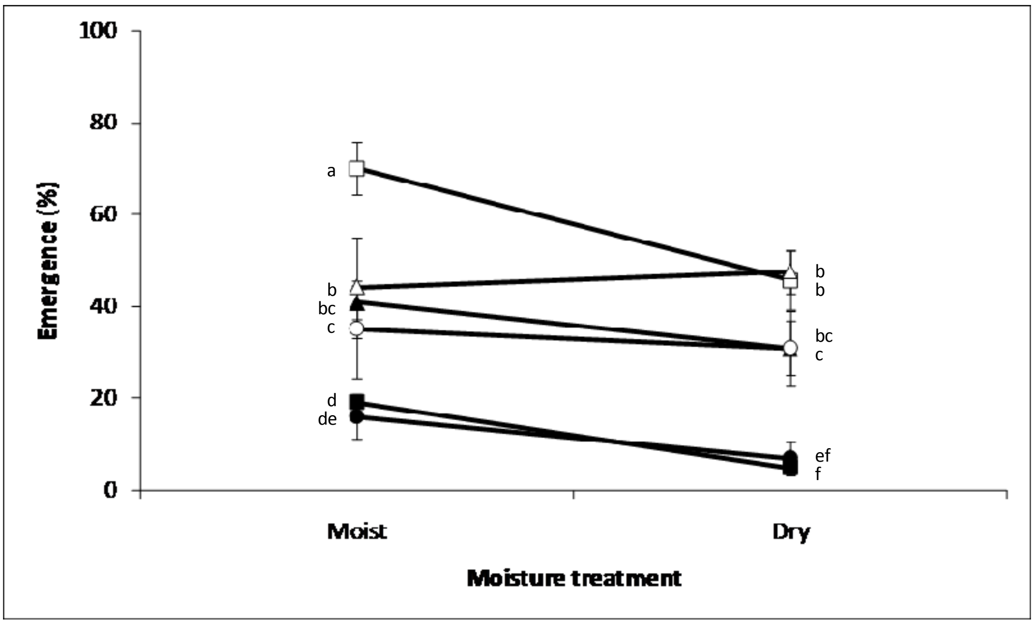


Figure 9. The percentage and standard error of seedlings which emerged in sand in moist and dry conditions over a period of 14 days. Pots were planted

with either naked (open symbols) or bracted (closed symbols) seeds of either Emergent *A. nummularia* (squares), Non-emergent *A. nummularia* (triangles), or *A. amnicola* (circles).

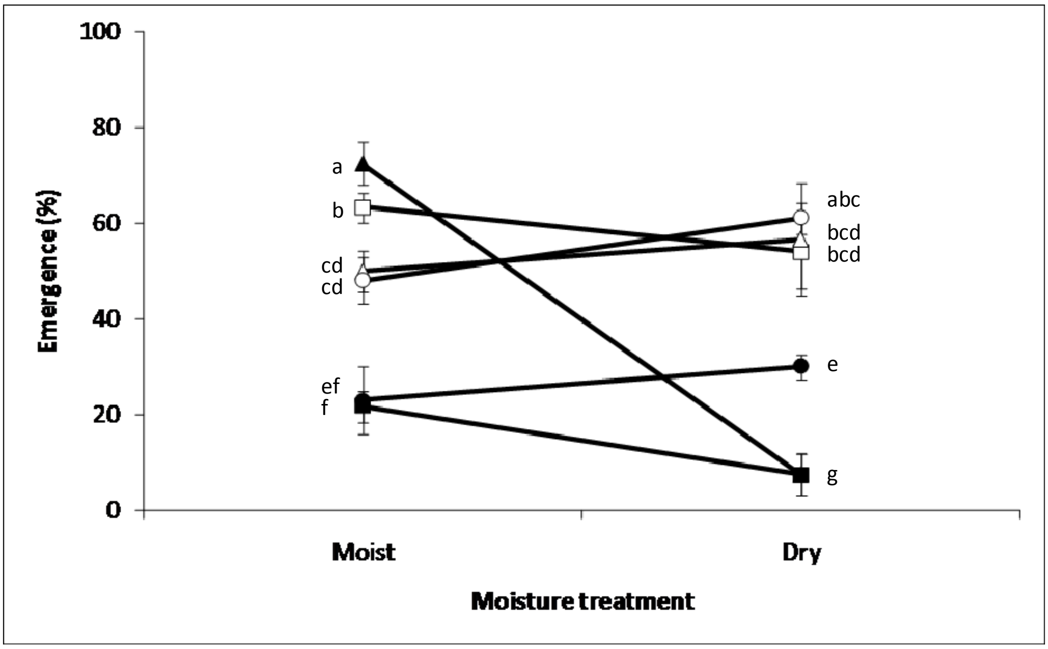


Figure 10. The percentage and standard error of seedlings which emerged in moist and dry loamy soil over a period of 14 days. Pots were planted with either naked (open symbols) or bracted (closed symbols) seeds of either Emergent *A. nummularia* (squares), Non-emergent *A. nummularia* (triangles) or *A. amnicola* (circles).

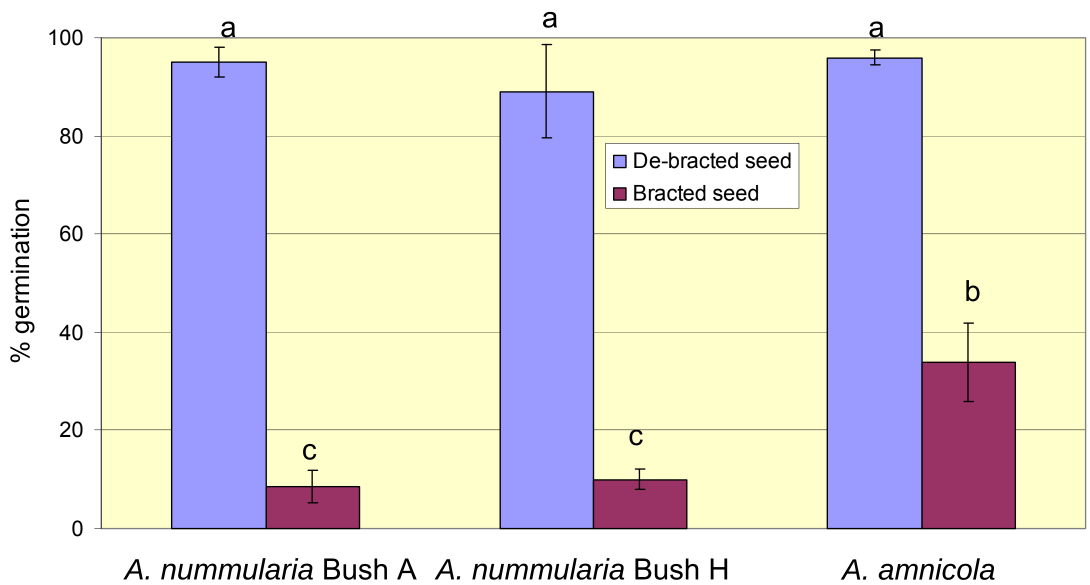


Figure 11. Germination percentage and standard error after 14 days in Petri dishes under laboratory conditions of bracted or de-bracted seeds of *A. amnicola* and *A. nummularia* types A (Non-emergent) and H (Emergent). Treatments with the same letter are not significantly different (*P*=0.05).

3.7.2. Imbibition of Emergent and Non-emergent *A. nummularia* types

Materials and methods

Seeds were prepared as in Section 3.6.1. Six groups of three petri dishes were plated with 10 bracted seeds of the two *A. nummularia* types, while another two groups were plated with 10 naked seeds per plate. All plates were lined with filter paper (Whatmans No. 1) to which 8 mL of tissue culture water was added. Plates were incubated at 20/10°C on a light/dark regime (Contherm 6000 CP). Bracted seeds were removed from the plates at 24, 48 and 72 hours after being imbibed. The seeds were then separated from the bracteoles and both were weighed. Both the bracteoles and seeds were dried at 107°C for 24 hours and re-weighed to determine the overall water content. Seeds from the naked seed treatment were weighed at 1, 2, 4, 8, 24, 48 and 72 hours after being imbibed; these were returned to Petri dishes after weighing. After 72 hours, the seeds were dried at 107°C for 24 hours and re-weighed to determine water content. Data were analysed as in Section 3.6.1.

Results

Overall, bracteoles of Emergent and Non-emergent *A. nummularia* types had higher water contents than both naked seeds and seeds enclosed within the bracteole, while naked seeds also tended to contain more moisture than those enclosed by bracteoles (Figure 11). Bracteoles of the Emergent type had a significantly higher water content than the Non-emergent type ($t = 0.02$). Seeds of the Emergent type enclosed within bracteoles also had higher water contents than the Non-emergent type after 24 hours of imbibition, but there were no significant differences between their water contents after 48 and 72 hours ($t = 0.26$ and $t = 0.52$, respectively). No significant differences were found between the water content of the two types of naked seeds ($t = 0.202$).

The percentage seed water content needed for germination was approximately 42% (Figure 12). Naked seeds germinated after approximately 20 hours. This was more rapid than seeds enclosed within bracteoles, which begin to germinate after approximately 48 hours for the Non-emergent type and after about 72 hours for the Emergent type.

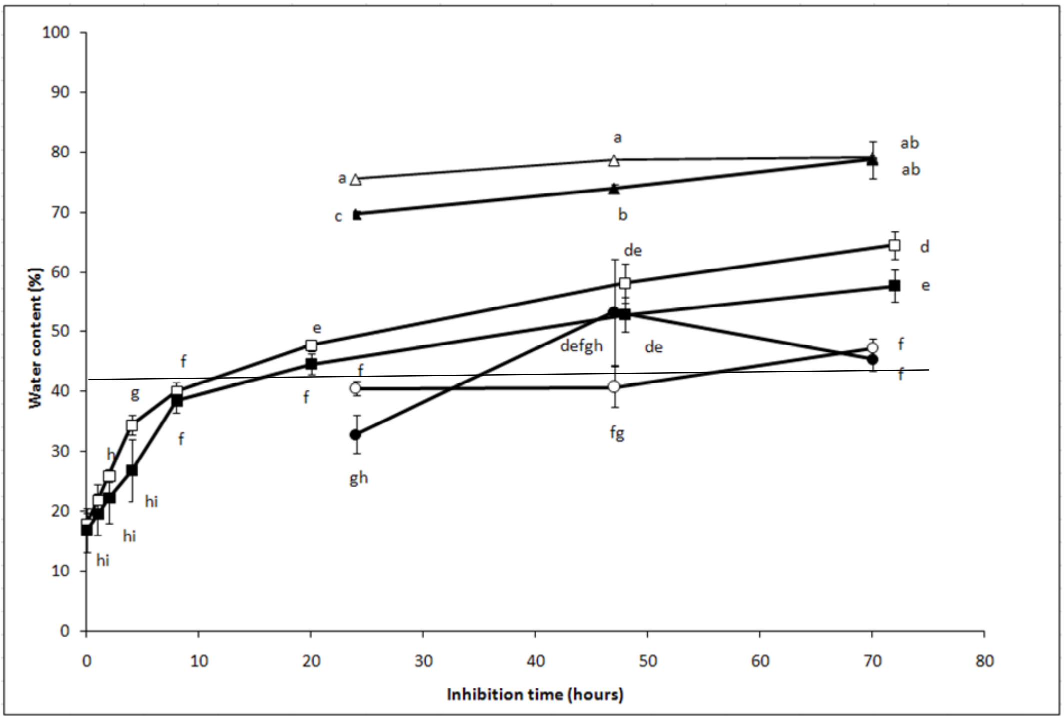


Figure 12. Percent water content and standard error of bracteoles (triangles), naked seeds (squares) and seeds enclosed by bracteoles (circles) over time after soaking in water. The imbibition times for both Emergent (open symbols) and Non-emergent (closed symbols) *A. nummularia* types are shown, along with the critical seed moisture content for germination (42% water content).

3.7.3. Elutes of *A. nummularia* bracteoles

Materials and methods

Seeds were prepared as in Section 3.6.1. Five grams of bracteoles of both the Emergent and Non-emergent *A. nummularia* types were crushed to a particle size of less than 500 µm, then soaked in 100 mL of tissue culture water for 24 hours. The solutes were filtered to remove particulates. The salt concentration of both elutes was measured using an EC meter and the molarity of both elutes calculated, assuming the salt was NaCl. Three replicates of 20 seeds of both *A. nummularia* types were placed on Petri dishes layered with filter paper (Whatmans No. 1). Each seed type was subjected to 10 mL of the two elutes and the plates incubated at 20/10°C on a diurnal light/dark regime (Contherm 6000 CP). The number of seeds germinated was recorded every two days until no further germination took place. Data were analysed as in 3.6.1.

Results

Salt concentrations in the elutes of the bracts of the Emergent and Non-emergent genotypes were similar, with the Emergent type containing 2.780 ppk of salt (47.57 mM if salt is NaCl) and the Non-emergent type containing 2.783 ppk (47.63 mM if salt is NaCl). The emergence rate of Emergent type seeds was higher than Non-emergent type seeds with both elutes (Figure 13). Furthermore, seeds of the Emergent type had a higher germination percentage in the Emergent type elute than in the Non-emergent type solution ($t = 0.02$), but there were no differences for the Non-emergent type seeds that germinated in the two elutes ($t = 0.374$).

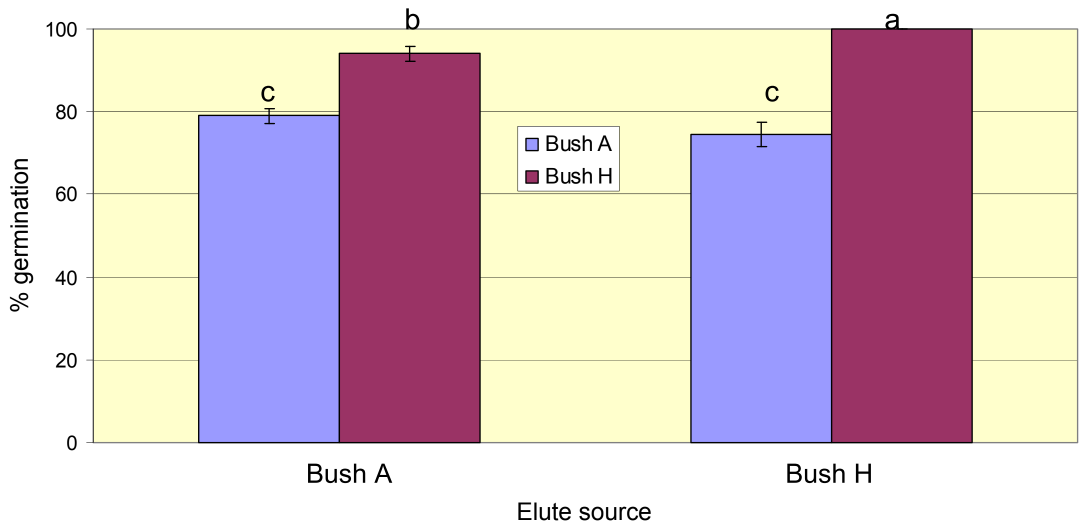


Figure 13. Germination percentage and standard error after 14 days in Petri dishes under laboratory conditions of seeds from *A. nummularia* Bush A (Non-emergent) and Bush H (Emergent) germinated in elutes made from Bush A and Bush H bracteoles. Treatments with the same letter are not significantly different (*P*=0.05).

General discussion - Emergence of bracted and de-bracted seed of Emergent and Non-emergent types

Bracted seeds of *A. amnicola* and *A. nummularia* tend to have a lower emergence rate than de-bracted seeds. This suggests that bracteoles either contain an inhibiting factor that retards germination or they need to absorb additional moisture to allow seeds to germinate. There also appear to be genotype differences within *A. nummularia* for ability to emerge from within bracteoles, with bracteoles of the Non-emergent type having a higher emergence percentage than naked seeds in the moist loam treatments. This may be because these bracteoles did not need to absorb as much water as those of the Emergent type before germination occurred.

Seeds of *A. amnicola* and the Non-emergent *A. nummularia* type had the same emergence rates in moist and dry soil, while the Emergent *A. nummularia* type had a higher emergence percentage in the moist soil. This suggests that Emergent type seeds require a higher moisture content than Non-emergent type seeds before germination can occur.

Bracteoles of A. *nummularia* appear to prolong the time taken for seeds to absorb enough water to germinate. This is suggested by the more rapid germination of naked seeds in the imbibition experiment. It appears that bracteoles need a water content of ~75% before the seed water content is high enough (~42% water content) to enable germination to proceed. The results also suggest a difference between the two types of *A. nummularia*, as the Non-emergent type required less moisture within the bracteole than the Emergent type for seeds to germinate. However, the water content of Non-emergent type bracted seeds declined after 48 hours, indicating this type of *A. nummularia* may be more drought prone than the Emergent type. These results suggest it is unlikely that differences in the testas of the seeds are responsible for the differences in germination between the two types of *A. nummularia*, as there were no differences in water content of their naked seeds. Instead it appears that a physical property of the bracteole is responsible for reducing the seed imbibition in bracted seed of the Emergent type.

Salt does not appear to be responsible for retarding germination in *A. nummularia*. This is because there was no difference between the salt concentrations in the two types of bracteoles, a result supported by experiments conducted by Stevens *et al.* (2006). Furthermore, the elute germination experiment suggests that chemical properties of *A. nummularia* do not affect the germination of Non-emergent type seeds. The small difference between the percentage of Emergent type seeds that germinated in the Emergent and Non-emergent type elutes may have been due to a structural property of the bracteole could retard germination, as it was observed that Emergent type bracteoles were more uniform in size and shape than Non-emergent type bracteoles.

In conclusion, bracted seeds of *A. amnicola* and *A. nummularia* tend to have a lower germination rate in the glasshouse than naked seeds in both loam and sand soils in dry and moist conditions, suggesting that the bracteoles have a role in regulating germination. It appears that bracteoles of *A. nummularia* need to have a moisture content of ~75% before the seeds inside can absorb enough moisture to germinate. As a result, it takes longer for bracted seeds to germinate than naked seeds. However, this may have an advantage under field conditions, as bracteoles of *A. nummularia* will only allow the seeds to germinate under optimal conditions, hence increasing the chance of seedling survival. Future research is needed to identify structural or chemical properties of the bracteole such as size, pH, the presence of hydrophopic chemicals/waxes and the presence of allelopathic substances which may be responsible for germination differences between Emergent and Non-emergent types of *A. nummularia*.

3.8. Seed priming to enhance germination

The germinability of a seed is regulated by its seed dormancy state and interactions with temperature, moisture and light. Priming (pre-germinating) seeds with water or germination stimulants has been successfully used for a range of species with plant signalling chemicals including karrikinolide (Flematti *et al.,* 2004), gibberellic acid (GA_3), benzoic acid (Senaratna *et al.*, 2003) and kinetin and salicylic acid (Senaratna *et al*., 2000). Kinetin is a cytokinin, a class of plant hormone that promotes cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement and auxiliary bud release and senescence. The function of this plant hormone requires auxins to be present. In contrast, salicylic acid is an inducer of systemic acquired resistance (SAR) to diseases in plants (Raskin 1992; Conrath *et al*. 1995) and is known to interact with plant respiration (Bourbouloux *et al*. 1998) and protein synthesis (Jin *et al*. 2000). Work by Dat *et al.* (1998) and Senaratna *et al.* (2003) shows that the use of salicylic acid increases thermo tolerance in seedlings. Unfortunately, the mode of action of these chemicals during germination remains unresolved. However, combined with other plant signalling chemicals, both kinetin and salicylic acid may provide additional support to improving germination, emergence and plant establishment.

Stevens *et al*. (2006) found under laboratory conditions GA_3 at 1000 ppm improved germination of *A. amnicola* and resulted in a four-fold increase in seedling emergence in the field. Priming with water significantly increased emergence percentage of *A. amnicola*, but had no effect on *A. nummularia* or *A. undulata*. Salicylic acid and kinetin improved the rate of emergence of all three species at various levels of salinity, while GA_3 also improved germination of *A. undulata*.

A series of experiments was conducted to examine whether plant signalling compounds could improve germination and emergence of the chenopod species *A. semibaccata*, *A. bunburyana*, *M. brevifolia* and *R. preissii*, particularly when physiological dormancy was active. Their effectiveness at different moisture levels was also examined.

3.8.1. Efficacy of plant signaling chemicals in the laboratory at critical moisture stress

Materials and methods

Seeds of all species used in these experiments were cleaned of inert material and dried for a minimum of 14 days at 16^{o}C and 25% relative humidity. Seed batches for all experiments had >98% viability, which was confirmed using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

In order to test the efficacy of plant signalling chemicals, the critical moisture stress level for germination was calculated for each species. Critical moisture stress for seed germination was defined as the water potential where germination levels are 75% of maximum germination. The effect of moisture stress on germination was investigated by supplementing germination media with polyethylene glycol (PEG, molecular weight 8000), equating to the solute potentials of 0, -0.25, -0.5, -0.75, -1, -1.25, -1.5 and -2 MPa. The osmotic potentials were calculated by equation 3, where x is the concentration of PEG_{8000} by %w/v (Michel and Kaufmann 1973).

$$\Psi_{PEG8000}\,(\text{MPa}) = -7.6049x2 - 33.025x + 4.83 \qquad (3)$$

Four replicates of 25 seeds of each species were plated into 90 mm vented Petri dishes containing a filter paper with 8 mL of each solute potential. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20^{o}C 12/12 hour light/dark constant incubator (TLMRIL model, Thermoline, QLD, Australia). This temperature was chosen as the osmolytic regression in equation (1) by Michel and Kaufmann (1973) is correlated with temperature. Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index was calculated using equations 1 and 2 in Section 3.5. All germination data were arcsine transformed and an ANOVA was conducted to compare the significance between treatments. Regressions and their equations in relation to the osmolytic gradient were plotted using CurveExpert 1.4. These equations were used to determine the critical moisture stress for each species at 20^{o}C.

Once the critical moisture stress levels had been determined, the effects of seven plant signalling agents and various combinations of them on improving germination percentage and germination rate were investigated for each species (Table 6). Seeds were primed in treatments for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Primed seeds were then left to bench dry for two days at 22^{o}C, 55% relative humidity, then transferred to a 16^{o}C, 25% relative humidity room to further dry for another four days. Four replicates of 25 seeds of each treatment were plated into 90 mm vented Petri dishes containing a filter paper to which 8 mL of the critical moisture stress solute potential for each species was added. Controls consisted of treated seed plated into Petri dishes with filter paper containing only water. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20^{o}C dark constant incubator (TLMRIL model, Thermoline, QLD, Australia).

Table 6. Germination enhancing chemicals used in experiments and their concentrations.

Seed enhancing agent	Concentration

Gibberellic acid (GA_3)	0.28 mM
Smoke water (SW)	1 %
Ethylene as ethaphon (Ethy)	20 mM
Karrikinolide (formerly Butenolide) (KAR_1)	0.67 µM
Potassium nitrate (KNO_3)	0.30 M
Kinetin (K)	0.05 mM
Salicylic acid (SA)	0.5 mM

Germination was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index across treatments were calculated using equations 2 and 3. All germination data was arcsine transformed and an ANOVA was conducted to compare significance between treatments. This enabled identification of optimum plant signalling chemical combinations (PSCC) for glasshouse and field trials.

Results

Table 7. Optimal plant signalling chemical combinations for improved germination of four chenopod species, using moisture stress to differentiate control (no treatment) and priming treatments. Degree of significance between control and priming treatments are denoted by asterisks where * = *P* <0.05, **= *P* <0.01 and * = *P* <0.001. Standard errors are in parenthesis.**

Species	Best treatment[a]	% germination after 14 days at CMS[b]		
		Control	Best treatment	*P*
Atriplex semibaccata	GA_3+SA	0 (0.0)	20 (2.3)	***
A. bunburyana	KAR_1+SA	6 (1.3)	24 (3.3)	***
Maireana brevifolia	GA_3+SA+K	2 (0.4)	11 (2.2)	**
Rhagodia preissii	GA_3+SA	4 (1.9)	29 (4.9)	***

[a]GA_3 = gibberellic acid (0.28 mM), SA = salicylic acid (0.5 mM), KAR_1 = karrikinolide (0.67 µM), K = kinetin (0.05 mM)

[b]Critical moisture stress, defined as the water potential that retards germination by 75% of maximum germination

Seven plant signalling chemical combinations were identified to be significantly effective in improving germination, using moisture stress to differentiate treatments (Table 7). These were GA_3+SA for *A. semibaccata*, KAR_1+SA for *A. bunburyana*, GA_3+K+SA for *M. brevifolia,* GA_3+SA+K for *R. preissii*. Under critical moisture stress conditions, the respective treatments improved germination in *A. semibacatta* from 0% to 20%, in *A. bunburyana* from 6% to 24%, in *R. preissii* from 4% to 29% and with a smaller effect in *M. brevifolia* from 2% to 11% (Table 7).

3.8.2. Efficacy of plant signalling chemicals in glasshouse and field conditions

Materials and methods

Three sets of treated seeds of *A. semibaccata*, *A. bunburyana*, *M. brevifolia* and *R. preissii* were prepared, one each for glasshouse and field trials and one for laboratory controls.

The following treatments were prepared: priming with H_2O, priming with the optimum chemical signalling chemical combination for each species (shown in Table 7, and no treatment (control).

Field trials were sown on 18 September 2008 into a free-draining sandy soil at the University of Western Australia Shenton Park Field Station. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha (i.e. 680 g/L), three and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeds were hand-sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a Latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored for emergence weekly for 30 days, then fortnightly over a 120 day period.

The laboratory controls were run on 10 September 2008 using the laboratory germination methodology described in previous sections. An alternating 26/13^{o}C 12/12 hour temperature regime was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark.

Glasshouse trials were sown on 21 May 2009 at 5-7 mm depth into 150 mm diameter pots containing free draining white sand to a 140 mm depth. Four replicates of 50 seeds of each treatment were used. An alternating 27/15^{o}C 12/12 hour temperature regime was used. Pots were irrigated daily and emergence was scored weekly over a 120 day period.

Results

Under the laboratory conditions, plant signalling chemical combinations improved germination significantly to controls in *A. bunburyana, M. brevifolia* and *R. preissii* (Table 8). Under these conditions, *M. brevifoli* and *R. preissii* displayed very strong physiological dormancy, which was overcome by the plant signalling chemical treatments. Under glasshouse conditions, chemical combinations again improved emergence significantly after 30 days in *M. brevifolia* and *R. preissii*. In this environment *A. semibaccata* also had increased germination, but *A. bunburyana* did not. However, there was a general lack of transfer of the successful laboratory and glasshouse treatments to the field environment, with only *R. preissii* having significantly greater emergence than the untreated control (Table 8). *A. bunburyana* and *M. brevifolia* failed to emerge over the trial duration.

Table 8. Percent germination in the laboratory after 14 days and percent emergence in the glasshouse and field after 30 days of seeds primed with water (H_2O), primed with the optimum plant signalling chemical combination (PSCC) shown in Table 7, compared with untreated controls. Standard error is shown in parenthesis. Within each environment, PSCC treatments within a species significantly different (*P* <0.05) from controls are denoted by an asterisk (*)

Species	Laboratory			Glasshouse			Field		
	Control	H_2O	PSCC	Control	H_2O	PSCC	Control	H_2O	PSCC
A. semibaccata	32 (4.0)	53 (9.6)	100 (0.0)*	24 (3.3)	30 (3.9)	46 (4.1)*			
A. bunburyana	12 (3.0)	27 (4.9)	39 (5.5)*	8 (1.0)	6 (1.3)	10 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)
M. brevifolia	8(0.0)	5 (2.7)	37 (5.3)*	4 (0.8)	5 (1.1)	12 (2.4)*	0 (0.0)	0 (0.0)	0 (0.0)
R. preissii	16 (4.6)	24 (8.0)	95 (3.5)*	22 (2.0)	28 (3.3)	48 (5.1)*	1 (1.0)	6 (3.2)	17 (3.5)*

Discussion

The use of plant signalling chemical combinations can improve the germination and emergence of perennial pasture species under laboratory and glasshouse conditions. However, there was a general lack of translation of this benefit to field emergence, except in species with significant physiological dormancy issues associated with the seed at the time of treatment. These results suggest that whilst the use of plant signalling chemicals are useful in improving germination percentage, rate and germination tolerance to moisture stress, there needs to be a greater understanding of the biological and environmental factors that reduce this benefit under field conditions.

The efficacy of plant signalling chemicals in glasshouse and field conditions could be dictated by several factors: chemical dependencies, dormancy status of seed and the stability of chemicals to temperature.

The use of kinetin may be an example of a chemical dependency. Here the efficacy of salicylic acid and kinetin to improve germination under moisture stress in laboratory conditions was a common theme across species. However, the use of kinetin in combination did not always work. Kinetin, a cytokinin, induces cell division but requires auxin to be present in order for it to be effective and the ratio of auxin to cytokinin is crucial during cell division (Mok and Mok, 1994). Auxins in plant tissues promote the production of ethylene, which along with GA_3, interact with light as a cue for germination. It is noteworthy that *A. semibacatta* and *R. preissii* did not require the presence of kinetin to benefit from priming and that both species have strong requirements for light in order to germinate. The efficacy of kinetin in species that did not respond significantly to the chemical may be due to a lack of auxin in the seed of these species.

The efficacy of chemicals on germination and emergence benefits can be affected by the dormancy status of seeds at the time of treatment. For example, the efficacy of GA_3 as a plant signalling chemical, particularly in a number of the chenopod species tested, appears dependant on the after-ripening status (Hilhorst and Karssen, 1992). Gibberellic acids (GA) are not directly responsible for breaking down dormancy but in combination with after-ripening relief, their inclusion aids the development of a GA-responsive system. GA biosynthesis in seeds occurs during seed imbibition and functions independently from dormancy (Karssen and Lacka, 1986). For example, the *R. preissii* seed in this experiment was four months-old, and likely to have been in a state of after-ripening. The priming combination of GA_3 and salicylic acid produced a higher germination percentage of *R. preissii*, even under field conditions. Low germination in the laboratory of untreated seed showed it to be highly dormant, but highly responsive to GA_3.

The potential to observe chemical hydro-priming benefits on seed germination and emergence is dependent on sampling frequency, experimental duration and optimal temperatures for germination and emergence. For many non-dormant species, the priming benefit is largely due to increases in the rate of germination and emergence. Hence, benefits from these chemicals occur within the first few days after imbibition and differences between priming and control treatments diminish with time.

Both *A. bunburyana* and *M. brevifolia* failed to emerge throughout the course of the field trial. Both species performed poorly under glasshouse conditions as well, with low emergence rates compared with germination percentages in laboratory controls. Previous trials of both species in irrigated plots at South Perth also performed poorly. In that trial however, considerable mortality (23%) in *M. brevifolia* occurred in the first four weeks while no emergence was recorded in the same species at Shenton Park. This indicates that high moisture contents are required for germination of *M. brevifolia* in sandy soils.

These experiments suggest field application of chemical hydro-priming appears feasible in *R. preissii* seed when it is highly dormant. While this appears to benefit emergence in such species, carryover to plant establishment and first year survival remains unresolved. The use of this enabling technology has potential but the lack of translation to the field environment suggests that there are other variables related to priming that need to be understood. Future research should focus on developing an understanding of seed and chemical integrity after priming and how this relates to the seedbed environment.

3.8.3. Post-treatment handling – seed drying after priming

While there are numerous reports on the effects of chemical priming on seed germination in the literature, very little emphasis has been placed on post-treatment (i.e. drying and storage) handling of treated seeds. This is a considerable oversight, as the method of drying and storage of primed seeds affects the degree and longevity of priming benefits and seed viability. Knowledge of these effects is particularly important if priming technology is to be used by the seed industry, as appropriate post-treatment handling methods seeds will need to be implemented to optimise treatment benefits.

Materials and methods

A. nummularia and *A. amnicola* were used in the first experiments, due to their positive response to priming treatments. Seeds were debracted and osmo-primed (-1.0 MPa, PEG 8000), with the addition of GA_3 (0.28 mM), kinetin (0.05 mM) and salicylic acid (0.5 mM), for 24 hrs. Seeds were washed and bench dried for one hour before being placed into drying chambers at 20°C. Three relative humidity (RH) chambers containing solutions of LiCl were used to give equilibrated 30, 50 and 80% RH levels. Seeds were stored for 18 days with the following drying treatments:

(i) 18 days at 30% RH;

(ii) 6 days at 50% RH, then 12 days at 30% RH; and

(iii) 6 days at 80% RH, then 6 days at 50% RH, then 6 days at 30% RH.

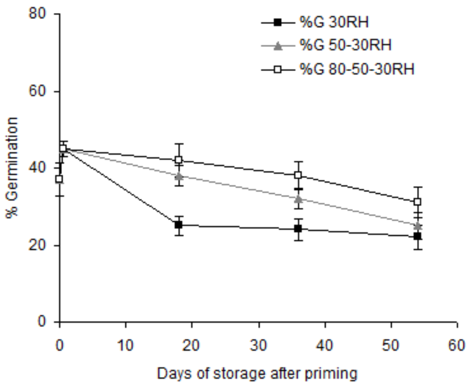
Seeds were sampled and germination tested at 18/7°C dark/dark on days 0, 1, 18 and 36 following treatment.

Results and discussion

Slow drying rates retained priming benefits longer for both *A. nummularia* and *A. amnicola*, with the slowest drying rate having greatest effect (Figure 14). These results indicate that at 20 °C storage, the priming benefits of the moderate drying rate treatment (6 days at 50% RH, followed by 12 days at 30% RH) tend to last for about 25 days in *A. nummularia* and 30 days in *A. amnicola*. The slow drying treatment (6 days at 80%, 6 days at 50% RH and 6 days at 30% RH) increased this to about 36 days in *A. nummularia* and more than 36 days in *A. amnicola*. Priming benefits were quickly lost with rapid drying at 30% RH. In *A. nummularia*, rapid drying also resulting in significantly reduced germination after 7 days.

These results have important implications for post-treatment handling of seeds primed with plant signalling chemicals. Rapid drying of fully hydrated seeds after priming may weaken or even rupture intracellular membranes in embryonic tissue, thus lessening seed shelf life.

A



B

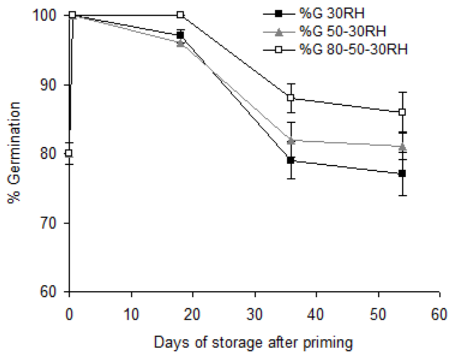


Figure 14. Germination percentage over time in *Atriplex nummularia* (A) and *A. amnicola* (B), following osmo-priming and drying under different relative humidity regimes.

4. Agronomy studies

4.1. Bracted vs unbracted seed – glasshouse and field emergence

Laboratory studies in Section 3.5 (Table 5) and from Stevens *et al.* (2006) showed that removing bracts from the seed substantially enhanced both germination percentage and germination rate of most Chenopod species. Two experiments were initiated to see if these results could be translated to improved emergence in small field plots and pots in the glasshouse.

4.1.1. Bracted and de-bracted seed of *A. amnicola* and *A. nummularia*

The first experiment involved bracted and de-bracted seed of *A. amnicola* and *A. nummularia* in the glasshouse.

Results

Glasshouse results showed that *A. nummularia* and *A. amnicola* differed in their ability to emerge in the de-bracted state (Figures 15-17). Under such conditions de-bracted *A. amnicola* seeds had significantly higher emergence than bracted seeds (Figure 15) - a similar result to that obtained under laboratory conditions (Section 3.5). In *A. nummularia*, however, the emergence of bracted seeds was significantly higher than de-bracted seeds, a result at odds with those from the laboratory. This result was examined further in field plots.

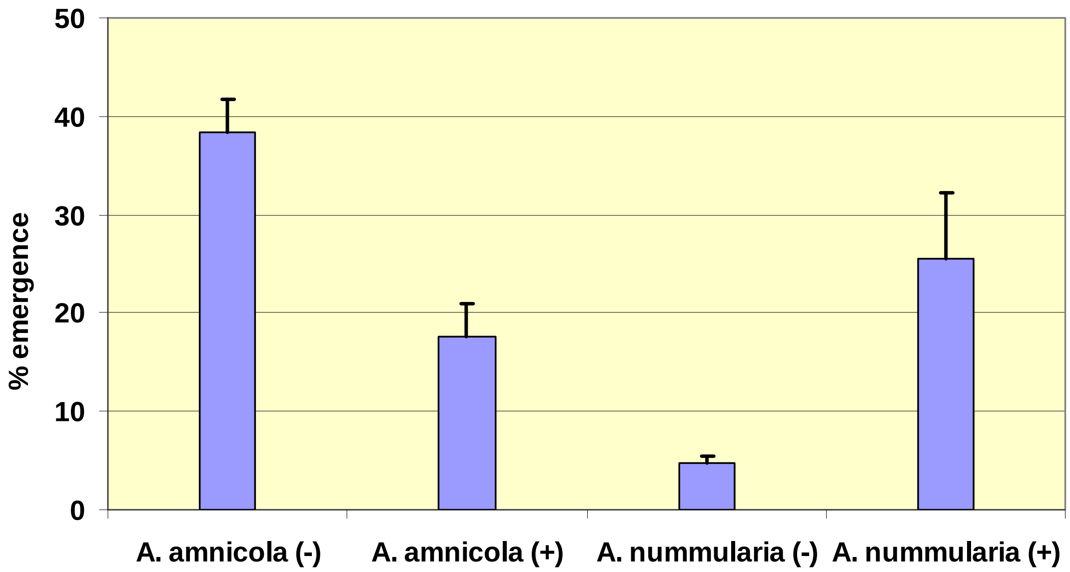


Figure 15. Seedling emergence of bracted (+) and de-bracted (-) *Atriplex amnicola* and *A. nummularia* 22 days after sowing into pots in the glasshouse.

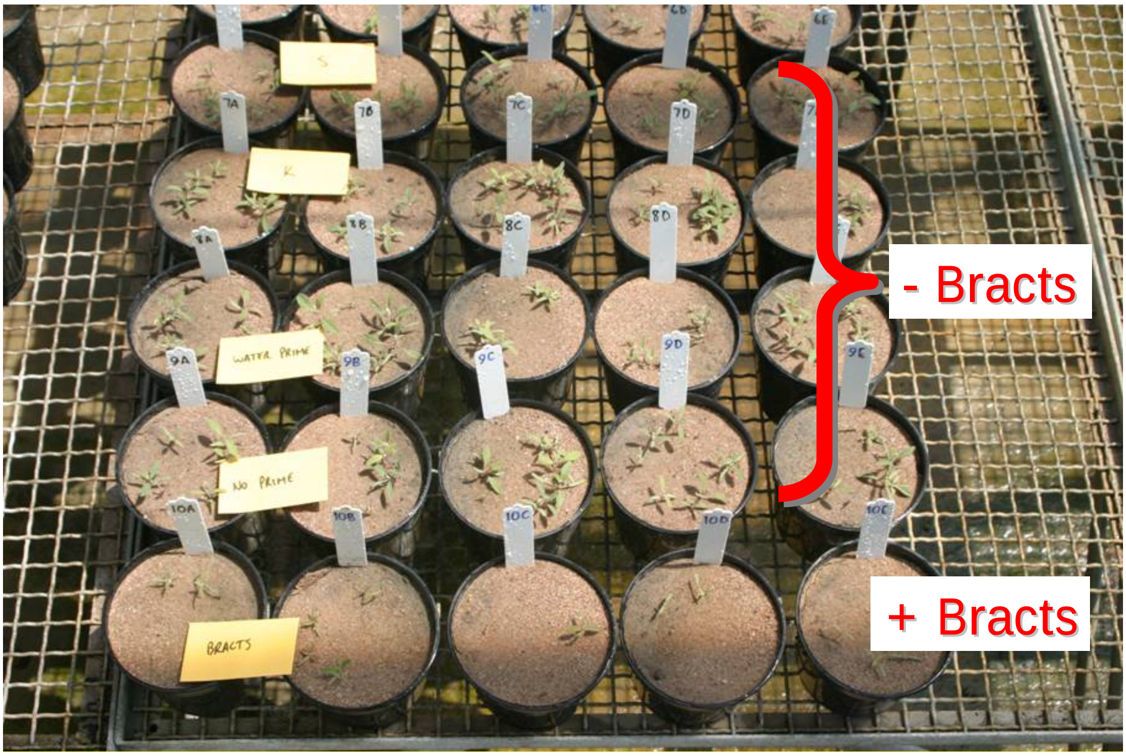


Figure 16. Emergence of bracted and de-bracted fruits of *Atriplex amnicola*

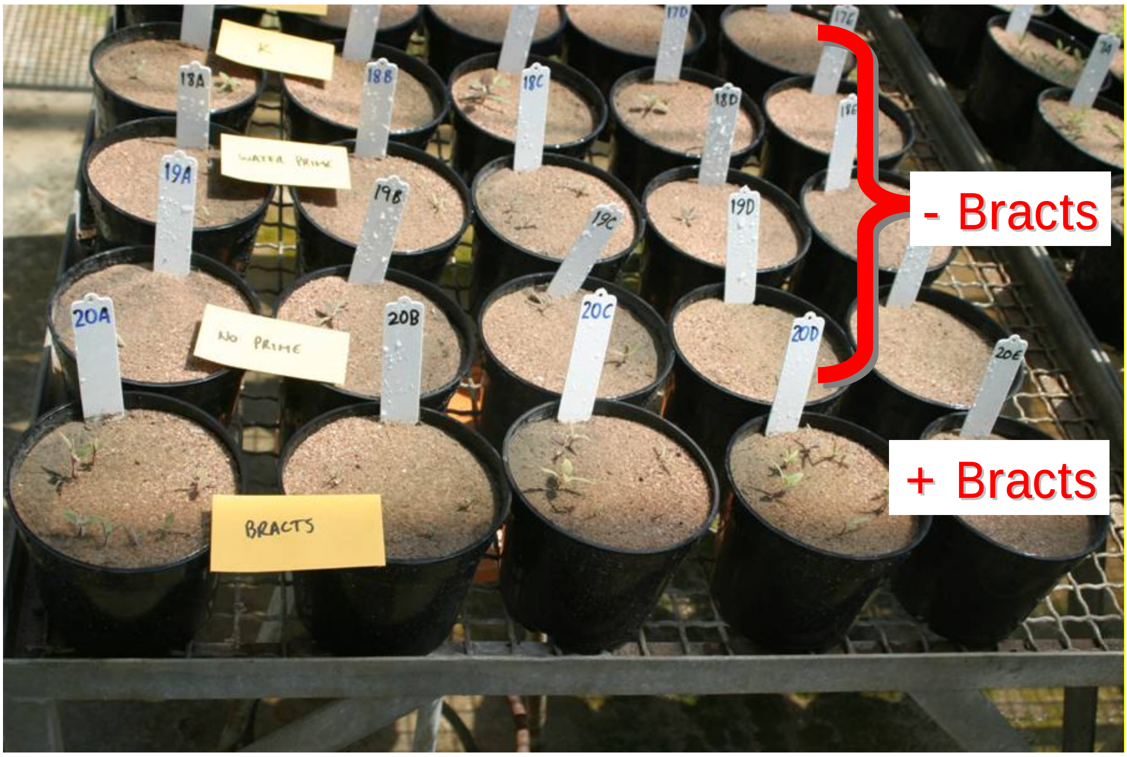


Figure 17. Emergence of bracted and de-bracted fruits of *Atriplex nummularia*

4.1.2. Bracted and de-bracted seed of A. cinerea, M. brevifolia and M. pyramidata

This experiment involved comparing the emergence of bracted and de-bracted seeds of *A. cinerea*, *M. brevifolia* and *M. pyramidata* in the field with germination in the laboratory.

Materials and methods

Field trials were sown on 23 October 2008 into a free-draining sandy soil at the University of Western Australia Shenton Park Field Station. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha (i.e. 680 g/L), three and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeds were hand-sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a Latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored for emergence weekly for 30 days.

The laboratory controls were run on 10 September 2008 using the laboratory germination methodology described in previous sections. An alternating 26/13^{o}C 12/12 hour temperature regime was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark. Germinating seedlings were scored twice weekly for 30 days.

Results and discussion

Under laboratory conditions, de-bracting markedly increased germination percentage of each species and increased the germination rate index of *A. cinerea* and *M. pyramidata* (Table 9). Without de-bracting no germination occurred for *M. pyramidata*, while only 1% of seeds germinated of *A. cinerea*.

However, the results for seedling emergence in the field trial were quite different to the laboratory germination results (Table 9). Emergence of bracted *M. pyramidata* was significantly higher than for de-bracted seed, in direct contrast to the laboratory results, where no germination occurred of bracted seeds. There were no differences between emergence of bracted and de-bracted seeds for *A. cinerea* or *M. brevifolia* in the field. Clearly other factors operate in the field to regulate seed germination. The role of bracts in these species requires further investigation.

Table 9. Field emergence and laboratory germination over 30 days following sowing. GRI = germination rate index (% germination/day) and ERI = emergence rate index (% seedlings/day). Values in parenthesis represent +/- 1 standard error.

Species	Treatment	Laboratory		Field	
		% germination	GRI	% emergence	ERI
A. cinerea	Bracted	1.0 (1.0)	0.1 (0.1)	3.5 (2.9)	0.1 (0.1)
	De-bracted	88.0 (3.6)*	5.7 (0.3)*	3.5 (2.4)	0.2 (0.1)
M. brevifolia	Bracted	21.5 (3.0)	0.8 (0.1)	9.5 (3.3)	0.7 (0.2)
	De-bracted	38.5 (5.4)*	2.0 (0.5)	2.0 (1.1)	0.1 (0.1)
M. pyramidata	Bracted	0.0 (0.0)	0.0 (0.0)	15.5 (6.6)	0.5 (0.2)
	De-bracted	93.0 (1.0)*	14.7 (0.2)*	7.5 (1.3)*	0.5 (0.1)

* denotes significant differences (P<0.05) between treatments within species.

4.2. Seeding depth

A small plot trial was conducted to examine the effect of seeding depth on emergence of *A. nummularia*. Population differences in ability to establish from sowing at different depths were also examined.

Materials and methods

The site consisted of a sandy soil at DAFWA headquarters in South Perth, Western Australia. Three seed sources were compared: seed collected from *A. nummularia* bushes on properties at Lake Grace and Tincurrin, WA, and a batch of commercially purchased seed in 2008.

Each plot comprised a row of 400 mm length, with furrows formed by hand to a depth of either 5 mm or 20 mm, into which 50 germinable seeds were sown and filled in by sand. The trial consisted of four replicates arranged in a randomised complete block design, giving a total of 24 plots. The trial was sown on 29 October 2008. Plots were irrigated by overhead sprinklers daily. Emerged seedlings were counted weekly for 7 weeks until the 8 December. Comparisons were made on percentage of viable seeds, which were determined using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999).

Results and discussion

Some seeds of each population emerged from a 5 mm sowing depth, but no seeds established from 20 mm (Figure 18). The other interesting observation was an apparent difference in ability to establish from a 5 mm sowing depth between different A. nummularia seed sources (Figure 18). This difference was upheld, even when differences in seed viability were taken into account. These results contrast with a nursery experiment by Vlahos (1997), who found that covering fruits of *A. amnicola* with 2 mm and 5 mm of soil decreased germination by 50% and 95%, respectively.

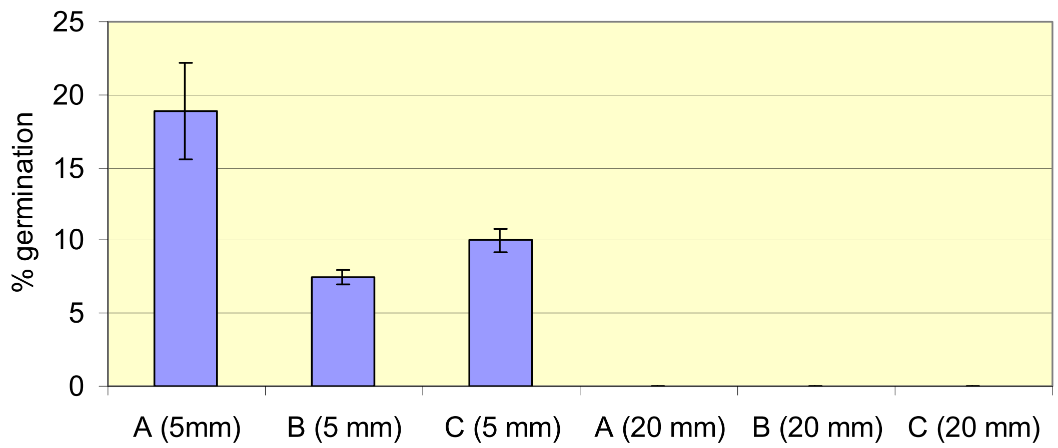


Figure 18. Percentage germination (of viable seeds) of three populations of *A. nummularia*, following sowing at a depth of either 5 mm or 20 mm in the field at South Perth. Populations A and B represent seed collected from bushes at Lake Grace and Tincurrin, respectively, while population C was commercially purchased seed.

4.3. Machinery configuration for sowing A. nummularia *and* A. amnicola

A trial was sown to determine the most appropriate seeding point configuration for direct sowing *A. amnicola* and *A. nummularia*. The use of depth wheels for sowing depth precision was also investigated.

Materials and methods

Field experiments were conducted on a mildly saline sandy loam site at Meckering, Western Australia sown on 14 August 2007. The site was sprayed with 2 L/ha Roundup® on 18 July 2007 and again one day prior to sowing. The insecticide Dominex® was also applied with the later spraying.

Chemically primed and de-bracted seeds of either *A. nummularia* and *A. amnicola* were sown, with sowing rates adjusted to 50 germinable seeds per metre (calculated from prior germination tests). Subsequent examination of the *A. nummularia* seed showed it to belong to subsp. *nummularia*.

Three different sowing tyne treatments were compared:

(i) t-boot knife points (TKP), which form a negligible furrow;

(ii) 8"-wide scarifying points cut in half, which enable placement of seeds on the side of furrows; and

(iii) 8"-wide scarifying points, which place seeds in the bottom of 50 -70 mm deep furrows

The wide point tynes in treatment (iii) were similar to what would often be used by a seeding combine. Trailing press wheels were used in each treatment. Depth wheels were also used in treatments (i) and (ii), but not in treatment (iii). Tynes were attached to an experimental cone seeder and changed for different sowing runs.

The trial contained four replicates arranged in a randomised block design. Seed was sown to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m bare ground buffers in between. Monthly establishment counts were conducted on emerged seedlings for the first 3 months, with a final count conducted six months after sowing.

Results and discussion

Rainfall and temperatures at Meckering are shown in Figure 19. Soil moisture conditions were favourable for germination. Seeds were sown into moist soil and 58 mm of rain fell over the following six weeks (until 30 September), with a further 23 mm in October (Figure 19). However only 47 mm fell over the five months between 1 November and 30 March.

Establishment of *A. nummularia* was favoured by sowing with knife points rather than with seed placed in furrows (Figure 20). Seedling counts on 6 September (23 days after sowing) showed that the t-boots resulted in 3.7 seedlings/m, whereas the half wide point and wide point (minus depth wheels) configurations had 1.2 and 0.1 seedlings/m, respectively. However, there was a decline in plant survival over summer in the rows sown by t-boots, while more plants emerged in late summer in rows sown by the wide point tynes, so that by the end of summer, establishment differences were much smaller.

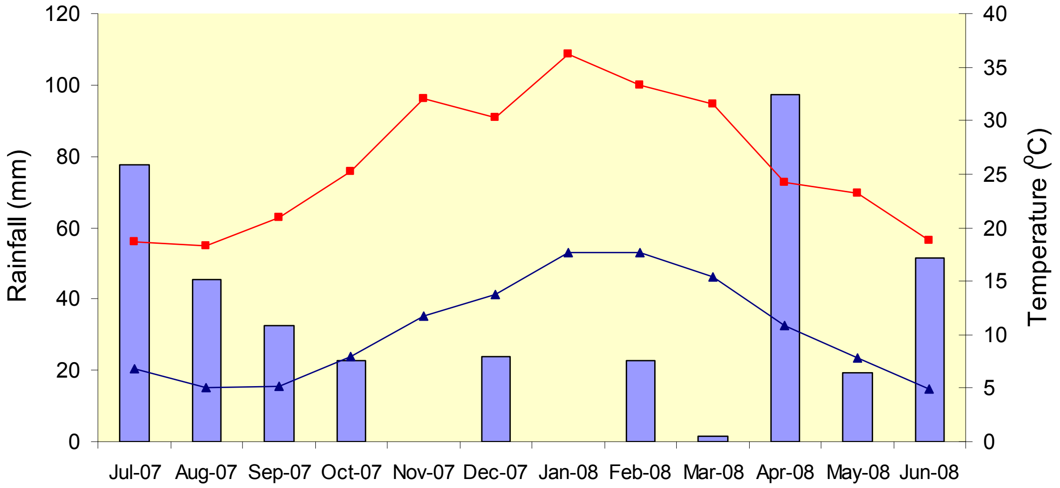


Figure 19. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2007 to 30 June 2008 at Meckering, WA (Data from the Australian Bureau of Meteorology).

Establishment densities of *A. amnicola* were much lower than for *A. nummularia*, indicating that its establishment in the field is more difficult than suggested from glasshouse results. The knife points and half wide point configurations gave the best initial establishment (Figure 21). However, late emergence during summer was again observed in rows sown by the wide point tynes, so that by the end of summer there were no differences in establishment density between tyne treatments.

Overall, the treatments involving a depth wheel had higher seedling emergence for both species. It is likely that the lower emergence from the widepoint tynes was at least partly due to insufficient control of sowing depth, as this treatment did not have a depth wheel, with seeds being placed too deep or too shallow.

Even though initial emergence was low, the formation of a furrow appears to have had some benefits for over-summer seedling survival. This is most likely attributable to its better ability to harvest rainfall for use by seedlings.

A possible constraint for *A. amnicola* is a requirement for warmer soil temperatures than those present in mid August at Meckering. *A. amnicola* originates from areas north of the agricultural zone, where winter temperatures are warmer. (The requirement of *A. amnicola* for warmer temperatures at germination was confirmed by the laboratory results in Section 3.1.) Later sowing times may be more successful, if sufficient soil moisture is present. More northerly sites, with warmer soil temperatures, may also lead to greater establishment success.

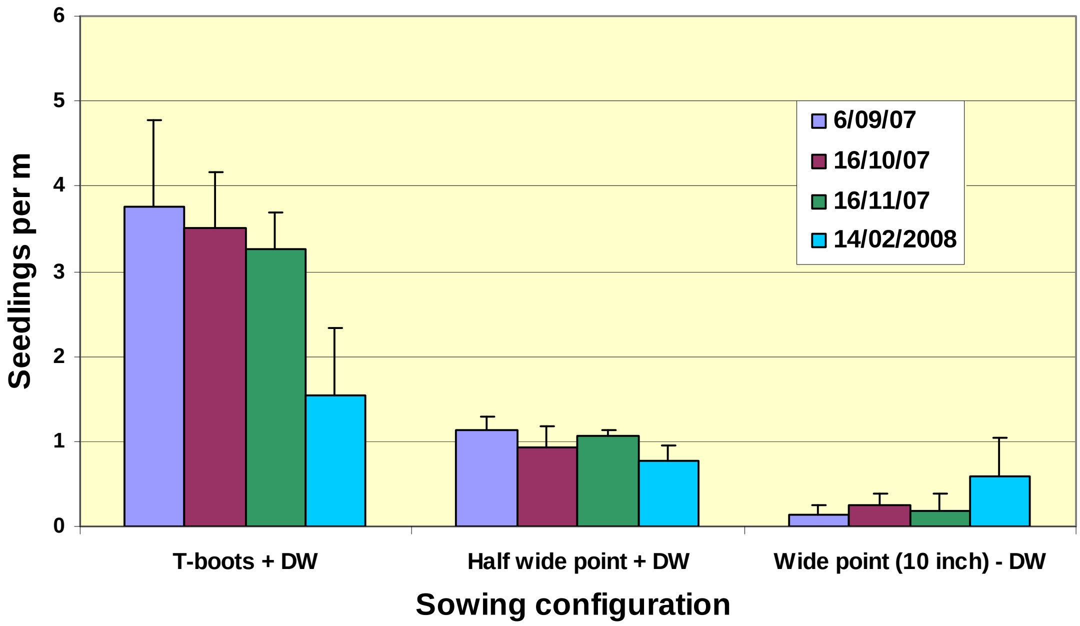


Figure 20. Established seedlings/m of row from four counts over time with *A. nummularia*, following sowing with t-boots, half-wide scarifying points and wide scarifying points (with no depth wheel).

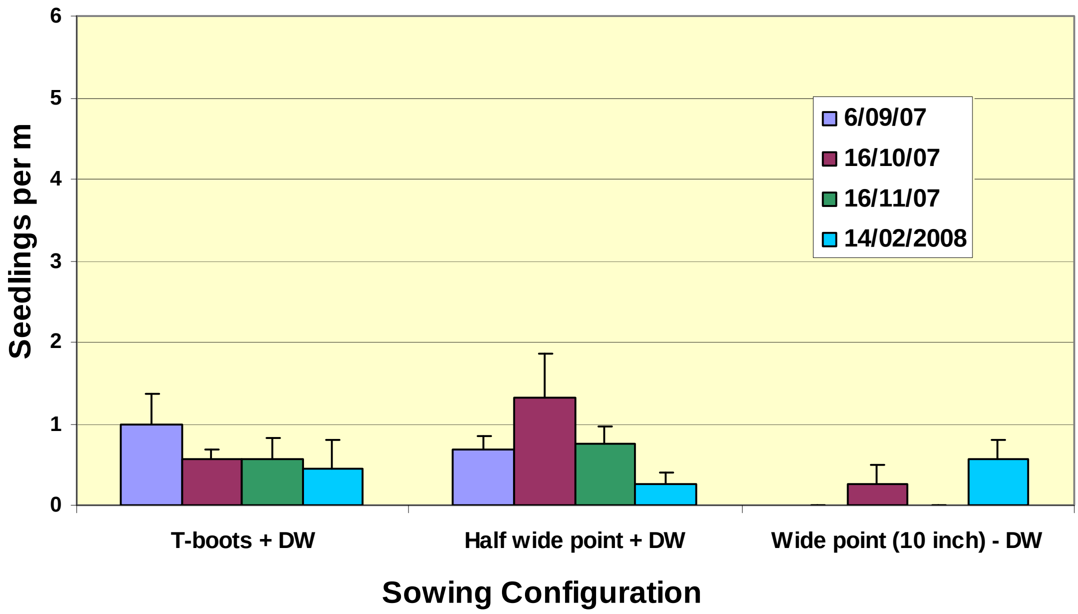


Figure 21. Established seedlings/m from four counts over time with *A. amnicola*, following sowing with t-boots, half-wide scarifying points and wide scarifying points (with no depth wheel).

This trial demonstrated for the first time, the possibility of successful direct seeding of *A. nummularia* in the field using conventional farm machinery (Figure 22).

Figure 22. The first successful direct seeding of *Atriplex nummularia* at Meckering, WA

4.4. Seed treatments to enhance establishment of A. nummularia *and* A. amnicola

The aim of this experiment was to determine whether seed enabling treatments that were successful in enhancing germination of *A. nummularia* and *A. amnicola* in the laboratory and glasshouse could be transferred to a mildly saline field site.

Materials and methods

This field experiment was conducted adjacent to the experiment on the mildly saline site at Meckering, WA described in Section 4.2 and site preparation was the same. The trial was sown on 14 August 2007. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

Seeds of *A. nummularia* ssp. *nummularia* and *A. amnicola* were either bracted or de-bracted (naked). These were subjected to three different priming treatments:

(i) primed with water and a combination of gibberellic acid (GA_3), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6;

(ii) primed with water alone; or

(iii) not primed.

Seeds were primed in treatments for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Primed seeds were then left to bench dry for two days at 22^{o}C, 55% relative humidity, then transferred to a 16^{o}C, 25% relative humidity room to further dry for another four days.

The trial contained four replicates arranged in a randomised block design and consisted of 48 plots divided into four banks of 12. Seed was sown using half 8”-wide scarifying points (HWP) to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m buffers. Sowing rates were adjusted to 50 germinable seeds per metre (calculated from prior germination tests). Establishment counts were conducted on emerged seedlings 39 days after sowing. Plant heights were measured 70 days after sowing.

Results and discussion

For *A. nummularia* bracted seeds had significantly higher seedling emergence across all treatments than de-bracted seeds (Figure 23). Seedling counts 39 days after sowing showed that of the bracted seed treatments, chemically primed seeds had the best overall emergence, with 16.4 seedlings/m, while un-primed and water primed seeds had 11 and 10 seedlings per metre, respectively. All de-bracted seeds had poor emergence (≤ 2 seedlings/m), although there was a small but non-significant response to water and chemical priming. There was no significant effect of the priming chemicals on plant height 70 days after sowing (Figure 23).

These results show that bract removal is detrimental to *A. nummularia* in the field, supporting the observations of Stevens *et al.* (2006) and the glasshouse results in Section 4.1.1.

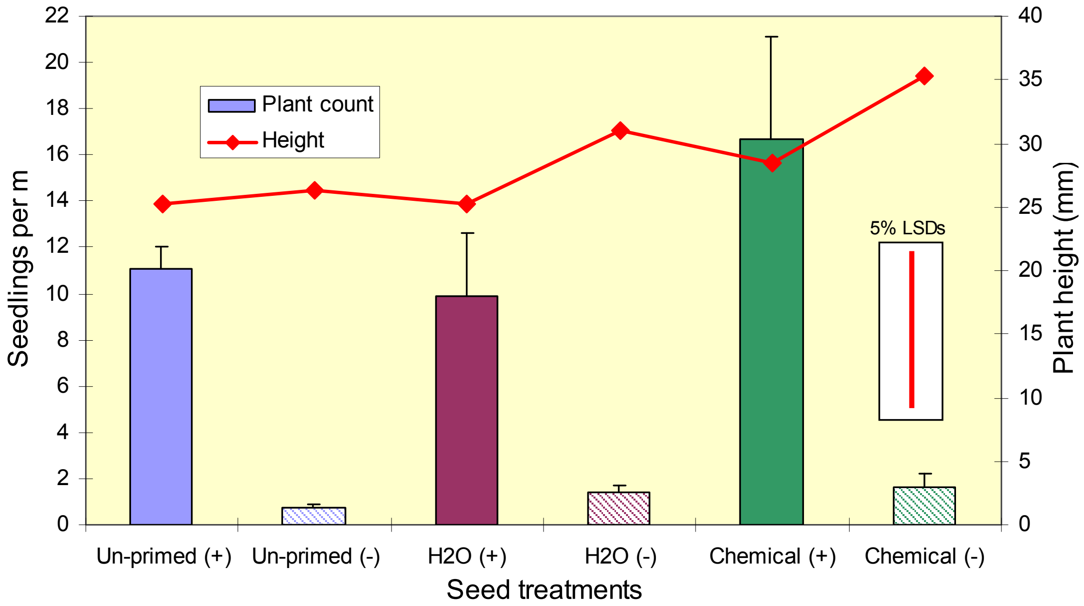


Figure 23. The effect of chemical priming (gibberrellic acid, salycilic acid and kinetin), water priming and un-primed seed on the emergence 39 days after sowing and height (mm) 70 days after sowing bracted (+) and de-bracted (-) *Atriplex nummularia* seed at Meckering, WA on 14 August 2007.

Seedling emergence of *A. amnicola* was much lower than *A. nummularia* for all treatments (Figure 24), reflecting the results of Section 4.2. Bracted seeds had a higher emergence than de-bracted seeds for each priming treatment, in contrast to the results of Stevens *et al*. (2006) and the glasshouse results in Section 4.1.1. These results suggest bract removal inhibits germination of *A. amnicola* in the field but enhances it in the laboratory or glasshouse. The reason for this discrepancy is not known.

Water-primed seeds had the highest seedling emergence per metre (1.5 seedlings/m), but there was no stimulus provided by the chemical priming (Figure 23). Chemical priming had a small positive effect on plant height 70 days after sowing for bracted seeds, but there was no difference for naked seeds (Figure 24).

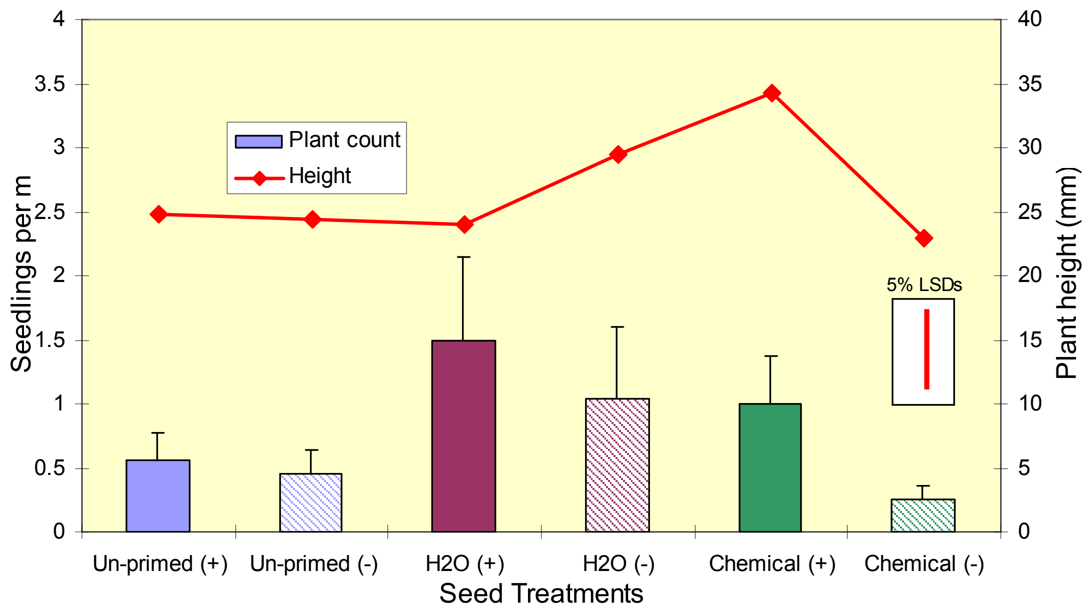


Figure 24. The effect of chemical priming (gibberellic acid, salicylic acid, and kinetin), water priming and un-primed seed on the emergence 39 days after sowing and height (mm) 70 days after sowing bracted (+) and de-bracted (-) *Atriplex amnicola* seed at Meckering WA on 14 August 2007.

4.5. Seed treatments to enhance establishment of A. undulata *and* M. brevifolia

The aim of this experiment was to determine whether seed enabling treatments in the laboratory and glasshouse that were successful in enhancing germination of *A. undulata* and *M. brevifolia* could be transferred to a mildly saline field site.

Materials and methods

This field experiment was conducted adjacent to the experiment on the mildly saline site at Meckering, WA described in Section 4.2 and site preparation was the same. The trial was sown on 14 August 2007. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

Three seed treatments were compared:

(i) un-primed bracted seed;

(ii) un-primed de-bracted (naked) seed; and

(iii) de-bracted seed chemically primed with a combination of gibberellic acid (GA_3), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6

The trial contained four replicates arranged in a randomised block design and consisted of 24 plots divided into four banks of six. Seed was sown using half 8”-wide scarifying points (HWP) to an approximate depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m buffers. Sowing rates were adjusted to 50 germinable seeds per metre (calculated from prior germination tests). Establishment counts were conducted on emerged seedlings at monthly intervals for three months.

Results and discussion

Seedling emergence of both species was poor, particularly for *M. brevifolia* (Figures 25 and 26). Initial seedling counts were higher for bracted seed of *A. undulata*, conforming to field results for *A. nummularia* and *A. amnicola*, and conflicting with the laboratory results of Stevens *et al.* (2006).

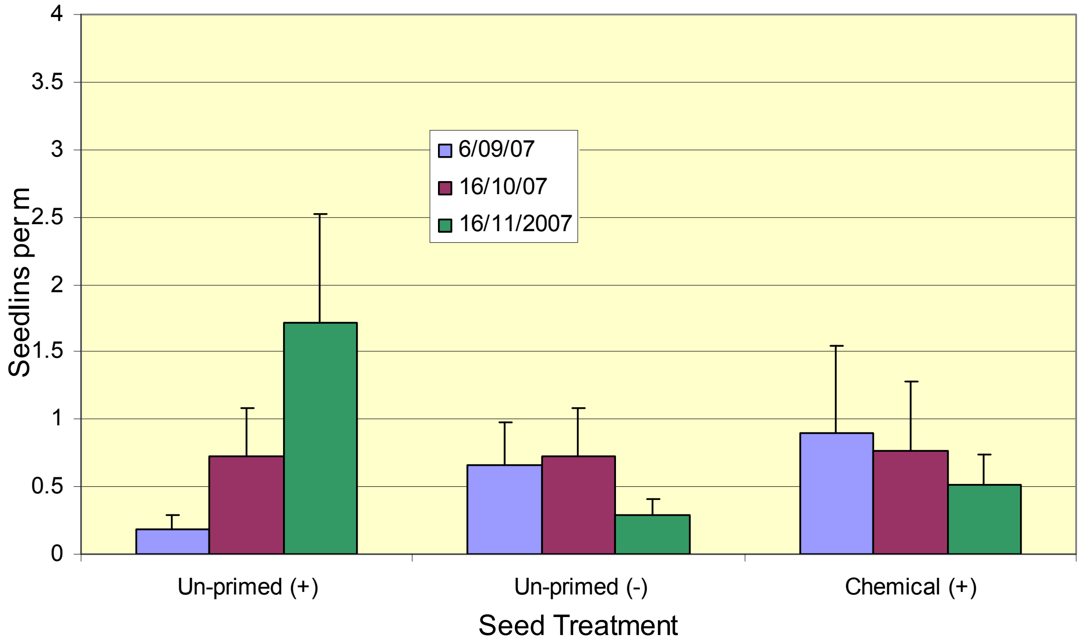


Figure 25. Seedling emergence from three counts over time of bracted (+) and de-bracted (-) un-primed seed and chemically primed de-bracted seed of *Atriplex undulata* sown at Meckering, WA on 14 August 2007.

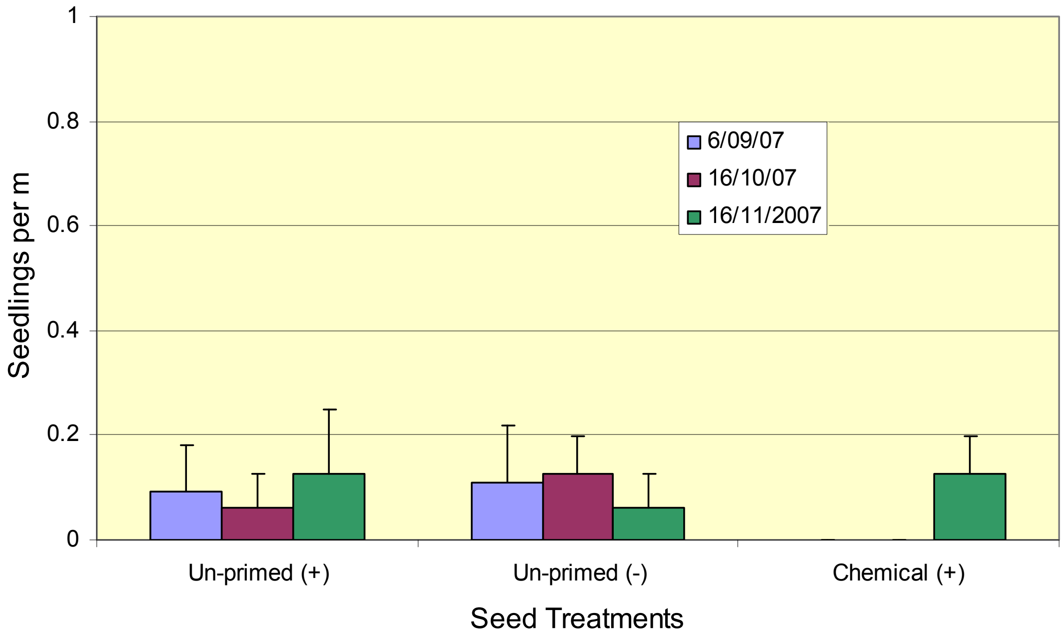


Figure 26. Seedling emergence from three counts over time of bracted (+) and de-bracted (-) un-primed seed and chemically primed de-bracted seed of *Maireana brevifolia* sown at Meckering, WA on 14 August 2007.

The very low emergence rates for both species, indicates that a much greater understanding of the environmental requirements for germination are required for these species before direct seeding can be contemplated. A major constraint, particularly for *M. brevifolia*, is likely to have been a requirement for warmer soil temperatures. This trial was sown in mid August, whereas the laboratory studies in Section 3.1 indicate *M. brevifolia* is more likely to germinate and establish after summer rains. Later sowing times and the use of other sowing configurations, such as t-boots, need to be further investigated for these species.

4.6. The effect of salinity and waterlogging on **A. nummularia** *and* **A. amnicola** *seedling emergence*

A trial was sown along a salinity transect at the Meckering site to determine the effect of salinity and waterlogging on the emergence of *A. nummularia* and *A. amnicola* seedlings. The effect of seeding point configuration on seedling emergence at different locations along the transect was also investigated.

Materials and methods

The site was located at Meckering within 100 m of the experiments described in Sections 4.2-4.4, but was located lower in the landscape. The site consisted of a 16 m saline transect, ranging from low salinity levels at the point of highest elevation to moderate levels at the point of lowest elevation. An EM38 survey was conducted on 16 October. EC_e values were derived from a calibration curve between EM38 readings (horizontal orientation) and are presented in Figure 27. The soil also contained more clay than in the other experiments and soil moisture was higher, particularly at the lower end of the transect. Site preparation was the same as described in Sections 4.2-4.4. Rainfall and maximum and minimum temperatures at Meckering are shown in Figure 19.

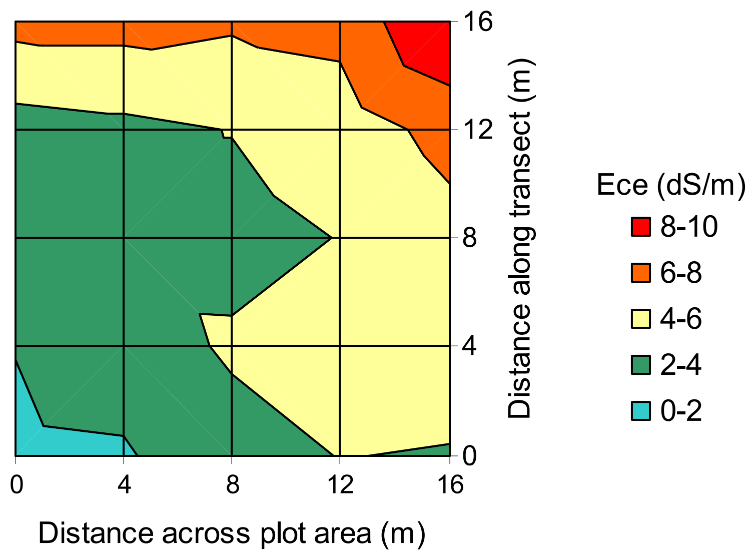


Figure 27. EC_e values (dS/m), estimated from a calibrated EM38 survey on 16 October 2007 along a salinity gradient at Meckering, WA.

De-bracted seeds of *A. nummularia* subsp. *nummularia* and *A. amnicola* that had been chemically primed with a combination of gibberellic acid (GA_3), kinetin (K) and salicylic acid (SA) at the rates shown in Table 6 were used.

Plots were sown on 14 August 2007 with a cone seeder using two sowing treatments:

(i) half 8”-wide scarifying points (HWP); and

(ii) t-boot knife points (TKP).

Results and discussion

The half wide point configuration gave a better establishment than the knife points, which was in contrast to the results in Section 4.2. One possible reason for this is that the soil in this experiment was more compact and did not have the same degree of furrow collapse (with consequent burial of seed) as in the sandier site. Furthermore, at this site the t-boots could not break into the soil, resulting in a much less favourable seed bed for germination.

Establishment of *A. nummularia* (Figure 28) and *A. amnicola* (Figure 29) did not appear to be affected by the salinity levels at the site. It is also important to note that most of the salt had been leached from the soil surface by this time of year. Of interest was the observation that plant numbers in this experiment were higher than in the adjacent experiment. For instance, *A. amnicola* and *A. nummularia* had seedling emergence of 3.8 and 6.3 plants/m, respectively, in the wetter transect area, compared to only 1 plant/m in the drier area of the adjacent trial. This supports laboratory results in Sections 3.5 and 3.6 that suggest bracts may act as a “moisture sponge” to prevent seeds drying out during the germination process.

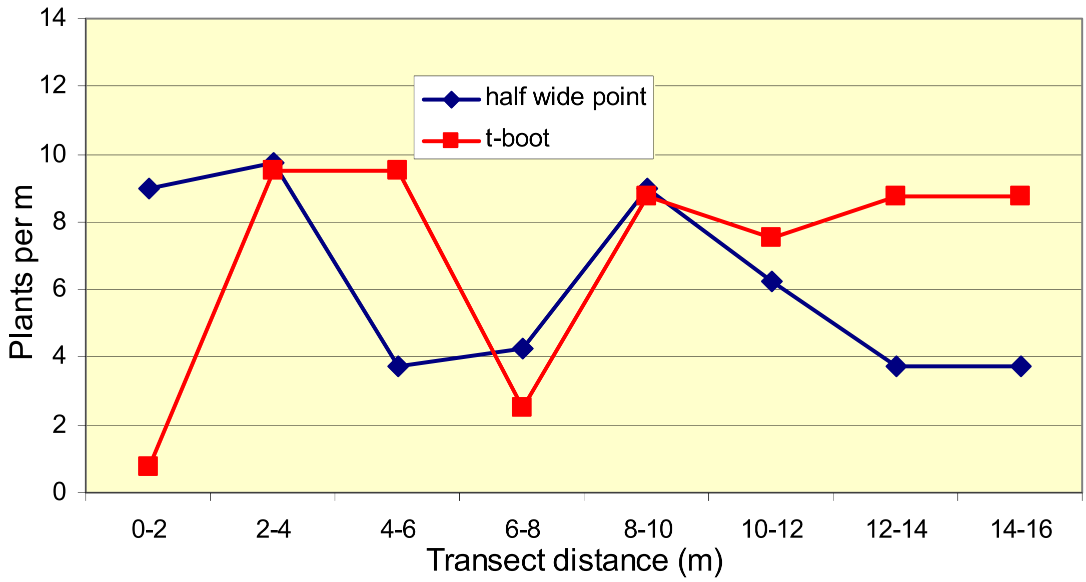


Figure 28. The effect of salinity on the germination of *Atriplex nummularia seed* (de-bracted and chemically primed) sown by either 1/2 wide point or t-boots sowing configurations at Meckering, WA. The trial was sown on 14 August 2007 and measurements were taken on 16 October (70 days after sowing).

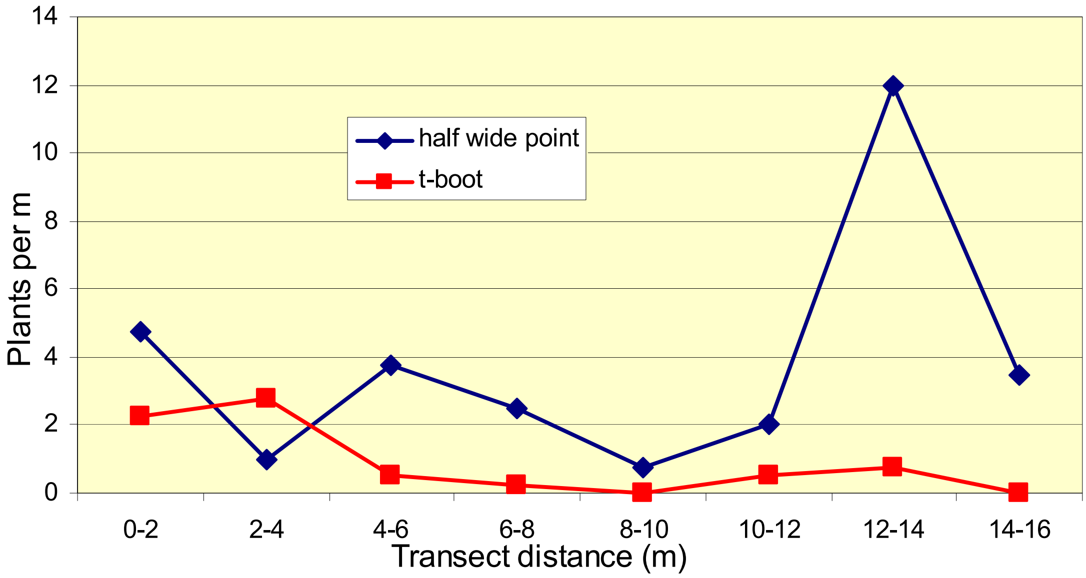


Figure 29. The effect of salinity on the germination of *Atriplex amnicola seed* (de-bracted and chemically primed) sown by either 1/2 wide point or t-boots sowing configurations at Meckering, WA. The trial was sown on 14 August 2007 and measurements were taken on 16 October (70 days after sowing).

5. Proof of concept direct seeding trials

The 2007 trial produced some very promising results, suggesting for the first time that direct seeding of *Atriplex nummularia* was feasible with conventional farm machinery, especially on a mildly saline, sandy duplex soil. However, the direct seeding of *A. amnicola*, *A. undulata* and *Maireana brevifolia* resulted in low establishment.

With these results in mind, it was decided to test the direct seeding of these species on other soil types and in different regions to further develop the establishment package. In particular, sites in the warmer regions of the agricultural areas of WA were used to investigate if warmer temperatures might have a bearing on establishment.

5.1. Success at Morawa

A trial was sown at Morawa located in the north-eastern agricultural region of WA. This site was chosen to represent the effect of warmer soil temperatures on chenopod establishment than in the 2007-sown trial at Meckering. The effect of a different soil type was also examined.

Materials and methods

The site was located near Morawa on a red loamy soil. Establishment of *Atriplex nummularia*, *A. amnicola*, *Maireana brevifolia* and *Rhagodia preissii* was examined. The *A. nummularia* seed was subsequently found to be of subsp. *nummularia*.

Chemically primed seeds of *A. nummularia*, *A. amnicola* and *M. brevifolia* and *Rhagodia preissii* were sown with their bracts intact, in contrast to the de-bracted seeds sown in the 2007 field trial. Sowing rates varied from 100 germinable seeds per metre for *A. nummularia* to 300 for *R. presseii* (calculated from prior germination tests) (see Table 10).

The trial was sown into moist soil on 21 July 2008 using a cone seeder with t-boot knife points, in combination with depth wheels. Trailing press wheels pressed seeds against the soil. The site was sprayed with 2 L/ha Roundup® on 20 June and again on the day of sowing. The insecticide Dominex® was also applied two days after sowing. Temperature probes were placed 1 cm beneath the soil surface to measure soil temperatures.

The trial contained four replicates arranged in a randomised block design. Seed was sown to a depth of 5 mm into two 5 m rows spaced 1.1 m apart, with 2 m bare ground buffers in between. Emerged seedlings were counted 8 weeks after sowing.

Results and discussion

The site received good rainfall through August (Figure 30), which provided ideal moisture conditions for germination.

Establishment rate of the *A. nummularia* seedlings was high, being 49% of that expected on the basis of the rate of germinable seed sown (Table 10, Figure 32). This confirmed that the 2007 success with direct seeding could be repeated on a loamy soil type.

Establishment of *Rhagodia presseii* was also successful, with 23% of the germinable seeds establishing as seedlings (Table 10, Figure 33). This was the first time that *R. presseii* had been successfully direct seeded, and demonstrated the feasibility of sowing this species with conventional farm machinery.

However, the *A. amnicola* and *M. brevifolia* sowings again resulted in poor establishment (Table 10). This was despite the Morawa site having mean maximum soil temperatures at 1 cm depth 2-5 °C warmer than the other sites (Figure 31). These results demonstrate that further information is needed on the constraints for direct seeding *A. amnicola* and *M. brevifolia*.

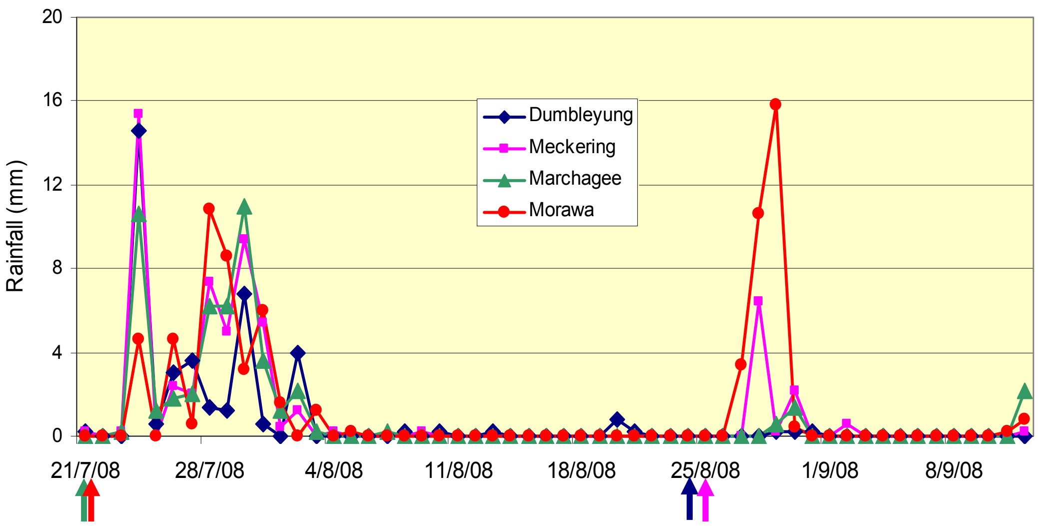


Figure 30. Rainfall (mm) received at Dumbleyung, Meckering, Marchegee and Morawa, Western Australia in 2008 between 20 July and 12 September. Arrows show time of sowing at each site.

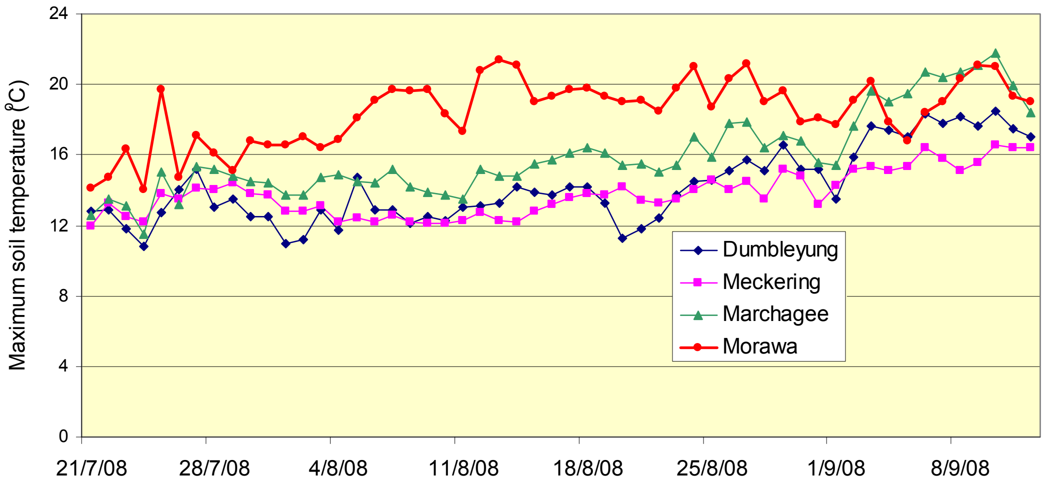


Figure 31. Maximum soil temperature (°C) measure 1 cm below the surface at Dumbleyung, Meckering, Marchegee and Morawa, Western Australia in 2008 between 20 July and 12 September.

Figure 32. Successful establishment of *Atriplex nummularia* at Morawa, WA following direct seeding

Figure 33. The first successful establishment by direct seeding of *Rhagodia preissii* at Morawa, WA

Table 10. Germination percentage, seeding rate, expected germination and observed establishment densities 8 weeks after sowing of *A. nummularia, A. amnicola, M. brevifolia* and *R. presseii* sown at Morawa, WA on 21 July 2008. The percentage of seedlings emerged to that expected is shown in parentheses.

Species	Germination (%)	Seeding rate (seeds/m)	Expected germination (seeds/m)	Observed seedlings/m
A. nummularia	39%	100	39	19 (49%)
A. amnicola	22%	130	29	2 (7%)
M. brevifolia	50%	150	75	0 (0%)
R. presseii	50%	300	150	34 (23%)

5.2. Effect of different seed sources on establishment of A. nummularia

Three additional field trials were sown in 2008 to test proof of concept of direct seeding *A. nummularia* using conventional seeding machinery on different soil types and locations.

Materials and methods

Sites were sown at Meckering (adjacent to the 2007 experiments described in Section 4.2), at Dumbleyung (350 km south-west of Perth) and Marchegee (300 km NNE of Perth). The Dumbleyung site consisted of a mildly saline grey loam clay, while the Marchegee site was a brown loam. Daily rainfall at each site between mid-

July and mid-September of 2008 is shown in Figure 30, while maximum soil temperatures 1 cm below the surface are shown in Figure 31.

A different seed source of A*. nummularia* to that sown at Meckering in 2007 and Morawa in 2008 was used. This was subsequently found to belong to subsp. *spathulata*. The seed also had low germinability. Even after retaining the heaviest 30% of fruits it only had 10% germinability.

Three seed treatments were examined:

(i) Bracted unprimed seed (graded to retain the heaviest 30% of seed);

(ii) Bracted unprimed seed (ungraded for seed weight); and

(iii) Bracted seed (graded to retain the heaviest 30% of seed) and primed with 0.05 mM of kinetin

Two different seeding point configurations (t-boot seeding knife points and half 8"-wide scarifying points) were also examined for each seed treatment. These were sown in combination with depth wheels for accurate seed placement, with trailing press wheels.

The trial contained four replicates arranged in a randomised block design. Seed was sown to a depth of 5 mm into two 8 m rows spaced 1.1 m apart, with 2 m bare ground buffers in between. Sowing rates of the graded seeds were adjusted to give a target of approximately 20 germinable seeds per metre.

Trials were sown at Marchegee on 21 July, Dumbleyung on 25 August and Meckering on 26 August. Sites were sprayed six weeks prior to sowing with 2 L/ha Roundup® for weed control and again on the day of sowing. The insecticide Dominex® was also applied with the later spraying. Emerged seedlings were counted 8 weeks after sowing.

Results and discussion

No seedlings of *A. nummularia* emerged at Dumbleyung, Meckering or Marchegee for any of the seed treatments or sowing configurations. The sites were moist at the time of sowing and conditions for germination appeared to be reasonable, apart from Dumbleyung, which received little rainfall for the three weeks post-sowing (Figure 30). Rainfall conditions for Marchegee were very similar to those at the successful Morawa site. Thus, it appears that poor moisture conditions for germination and establishment were not the main reasons for the seeding failures at Dumbleyung, Meckering and Marchegee.

The greatest difference between the sites appeared to have been the source of *A. nummularia* seeds. Subsequent examination of the seeds sown at Dumbleyung, Meckering and Marchegee showed them to belong to subsp. *spathulata*. This compared with subsp. *nummularia* for the seeds sown at Morawa in 2008 and at Meckering in 2007. Thus, it appears there are subspecies differences within *A. nummularia* for ability to establish from direct seeding in August-September, with subsp. *nummularia* apparently being better suited.

6. Refining the establishment package for A. nummularia

Previous experiments at Meckering in 2007 and Morawa in 2008, described in Section 5, showed that *A. nummularia* could be successfully established from seeds using conventional seeding equipment. Related trials also suggested subspecies differences in ability to establish from seeds, with subsp. *spathulata* being more difficult to establish from seed than subsp. *nummularia*. Other glasshouse and laboratory experiments described in Section 3.4 showed genotype differences within ssp. *nummularia* for ability to establish. Laboratory experiments, described in Section 3.7, also showed that germination could be stimulated by priming seeds with chemical signalling compounds, such as kinetin, although this had not previously been translated successfully to the field.

Two trials in different environments and soil types were sown to confirm previous findings and to further refine the direct seeding package for *A. nummularia*. The following hypotheses were tested:

1. *A. nummularia* can be successfully established from seed using conventional seeding machinery;
2. The use of priming with the chemical signalling compound, kinetin, enhances germination in the field;
3. Subspecies *nummularia* can be more readily established from seed than ssp. *spathulata*;
4. Genotypes of subsp. *nummularia* differ in their ability to establish from seed.

Materials and methods

Four *A. nummularia* populations were compared with two seed treatments: primed with kinetin and unprimed. Three of the populations were of subsp. *nummularia*, with the fourth being a commercial seed source of subsp. *spathulata*. Two genotypes of subsp. *nummularia* were obtained from the property of Michael Lloyd at Pingaring. One of these (Emergent type) was obtained from a bush (Bush H) that was observed to have a high density of seedling recruits surrounding it, which was subsequently found to have high establishment rates in the glasshouse. The other (Non-emergent type) was from a bush (Bush A) with no seedling recruits, which was found to have low establishment rates in the glasshouse. Further details of the Emergent and Non-emergent types are given in Section 3.4. The third subsp. *nummularia* genotype was a commercial source of cv. De Koch.

Seeds with intact bracts were separated from any debris with a vacuum aspirator and x-rayed using a Faxitron MX-20 x-ray machine. Any seeds lacking embryos were removed from the sample by hand. The remaining seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for experiments.

Primed seeds were soaked in 0.05 mM kinetin for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Seeds were then left to bench dry for two days at 22°C and 55% relative humidity and were then transferred to a 16°C, 25% relative humidity room to further dry for another four days.

Trials were sown on a well-drained loamy soil 15 km east of Mingenew (29°13’02”S, 115°35’40”E) and on a saline, waterlogged, sandy loam 13 km south of Wagin (33°25’35”S, 117°22’35”E). Weed control consisted of a double knockdown with glyphosate (17 July and 19 August at Mingenew, and 3 August and 31 August at Wagin).

Plots consisted of 8 m single rows, into which 200 seeds of each treatment were sown unto uncultivated soil using an experimental cone seeder. Treatments were replicated four times in a randomised block design. Seeds were sown 5-10 mm below the surface into 3 cm deep furrows. Depth wheels were used to ensure precise seed placement. Seeds were pressed into the soil surface with press wheels. Sowing dates in 2009 were 27 August at Mingenew and 7 September at Wagin. The Mingenew site was sprayed with Talstar and Lorsban on 3 September, while the Wagin site was sprayed with Talstar on 14 September.

Seedling counts were conducted at Mingenew on 29 September (34 days after sowing) and at Wagin on 7 October (30 days after sowing). Analyses of Variance were conducted on treatment means to determine whether establishment densities differed between genotypes and priming treatments.

Results and discussion

Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 are shown for Wagin in Figure 34 and for Mingenew in Figure 35. Germination conditions were very favourable at Wagin. The soil surface was very moist at sowing and remained moist until mid-October, with 51 mm of rain falling in the five weeks after sowing (Figure 34). Conditions for germination were less favourable at Mingenew. At the time of sowing, the soil was dry in the top 2 cm, but seeds were placed into moisture in the bottom of furrows (and pressed into moisture with press wheels). However, only 34 mm of rain fell in the five weeks after sowing and the soil surface was intermittently dry during this period, as temperatures increased rapidly (Figure 35).

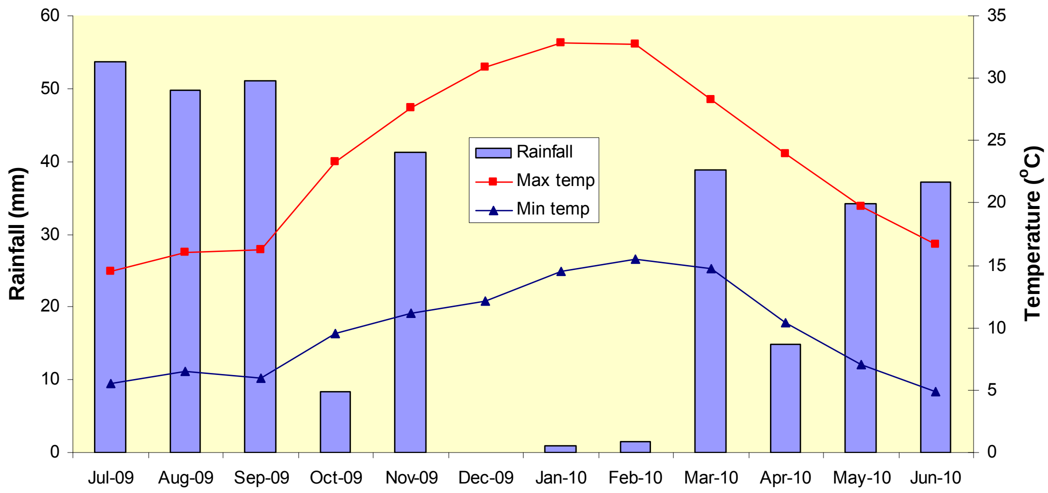


Figure 34. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Wagin, WA (Data from the Australian Bureau of Meteorology).

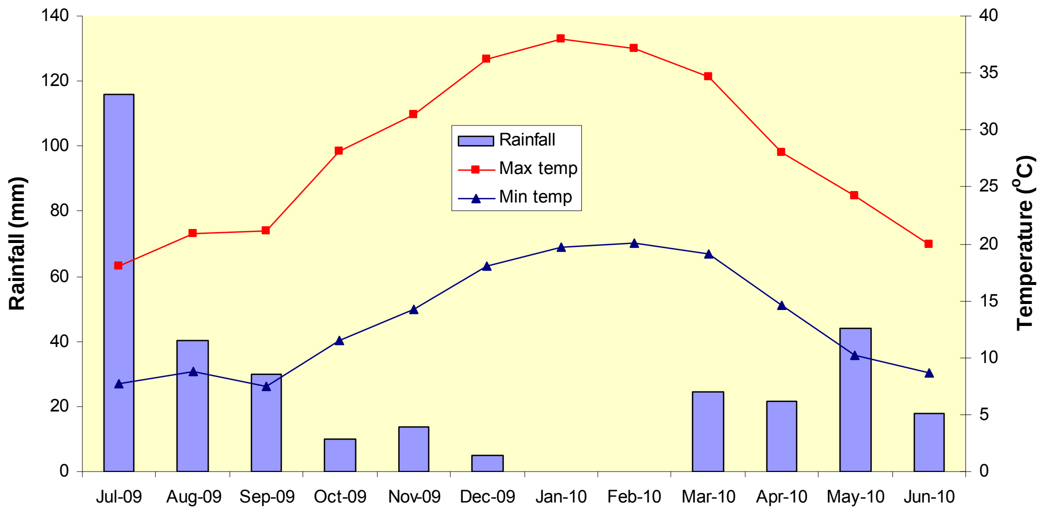


Figure 35. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Mingenew, WA (Data from the Australian Bureau of Meteorology).

Photographs of the Wagin and Mingenew sites are shown in Figures 36 and 37, respectively. Seedling counts per metre of row are shown for Wagin in Table 11 and for Mingenew in Table 12. Establishment was much higher at Wagin. Significant differences occurred between saltbush populations at both sites, with the Emergent type having significantly higher plant numbers than the Non-emergent type. Subspecies *spathulata* also had very low establishment at both sites. There were no significant effects of priming, and no significant genotype x priming interactions at either site.

These results confirm that *A. nummularia* can be established by direct seeding using conventional seeding equipment. A precise sowing depth of 5-10 mm appears to be critical for success. Use of kinetin as a seed priming agent appears to be less important.

These trials confirmed the unsuitability of ssp. *spathulata* for direct sowing. An extremely low proportion of seedlings emerged, in spite of them having high viability. The reason for the large difference between subspecies in ability to establish from direct seeding is unknown and requires further investigation to better understand the mechanisms.

These results confirm previous glasshouse indications of genotypic differences within subsp. *nummularia* for ability to establish from seed, with the Emergent type having markedly higher emergence. Seed of the Emergent type has been forwarded to the Future Farm Industries CRC saltbush breeder, for inclusion of ability to establish from seed as a breeding objective. These results also suggest that a wider examination within and between *A. nummularia* populations is likely to find even larger differences for ability to establish from direct seeding.

Establishment densities of the best treatment (Un-primed, Emergent type) at Wagin resulted in more than 4 plants/m (Table 11). This is clearly denser than the 1-2 plants/m required for long-term stands and indicates that the sowing rate was higher than required. This implies that sowing rates for this seed batch could have been reduced to around 10 seeds/m for a satisfactory establishment.

Table 11. Seedlings per metre of *Atriplex nummularia* populations with and without seed priming with 0.05 mM kinetin at Wagin, WA

Population	Primed	Un-primed	Mean
de Koch	0.91	1.19	1.05
Emergent type	2.34	4.19	0.37
Non-emergent type	2.25	1.66	1.95
ssp. *spathulata*	0.00	0.16	0.08
Mean	1.38	1.80	1.59
Genotype difference	*P*<0.001		
l.s.d.	1.217		
Priming difference	Not significant		
Genotype x priming differences	Not significant		

Figure 36. High seedling density of the "Emergent" genotype of old man saltbush following direct seeding at Wagin on 7 September 2009. Photograph taken on 24 November 2009.

Table 12. Seedlings per metre of *Atriplex nummularia* populations with and without seed priming with 0.05 mM kinetin at Mingenew, WA

Population	Primed	Un-primed	Mean
de Koch	0.47	0.09	0.28
Emergent type	1.22	0.91	1.06
Non-emergent type	0.16	0.03	0.09
ssp. *spathulata*	0.03	0.13	0.08
Mean	0.47	0.29	0.38
Genotype difference	*P*<0.001		
l.s.d. (*P* = 0.05)	0.474		
Priming difference	Not significant		
Genotype x priming differences	Not significant		

Figure 37. Establishing seedlings of the "Emergent" genotype of old man saltbush following direct seeding at Mingenew on 27 August 2009. Photograph taken on 1 December 2009.

7. Comparison of direct seeding techniques for A. nummularia

A demonstration trial was conducted, in collaboration with the Saltland Pastures Association, to compare direct seeding of *A. nummularia* using an experimental cone seeder with a commercial "Mallen" niche seeder.

Materials and methods

The trial was located on a saline, sandy loam, prone to waterlogging, 25 km south of Wickepin (32°59'59"S, 117°33'11"E). A weed-free seedbed had been prepared at the site with a double knockdown with glyphosate (2L/ha) six and two weeks prior to sowing.

Two unreplicated 60 m strips, located 70 m apart, with each strip containing two rows, were sown on 7 September 2009. One strip was sown with an experimental cone seeder, which placed seeds 5-10 mm below the surface into 3 cm deep furrows. Depth wheels were used to ensure precise seed placement and trailing press wheels ensured good seed-soil contact. The other strip was sown by a commercial saltbush seeding contractor using a "Mallen" niche seeder (see Figure 38). Sowing rate of the cone seeder was 33.6 seeds/m, while the Niche seeder was configured to deliver 25 seeds in each "placement", located 2 m apart.

The same commercial seed batch of cultivar de Koch (subsp. *nummularia*) was sown in both machines. Seeds with intact bracts were separated from any debris with a vacuum aspirator and x-rayed using a Faxitron MX-20 x-ray machine. Any seeds lacking embryos were removed from the sample by hand. The remaining seeds were checked for viability using the tetrazolium staining method described by the International Seed Testing Association (Anon. 1999). Only confirmed viable seeds were used for experiments.

One row in each strip was sown with seed primed with kinetin, while the other row was sown with unprimed seed. Primed seeds were soaked in 0.05 mM kinetin for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Seeds were then left to bench dry for two days at 22°C and 55% relative humidity and were then transferred to a 16°C, 25% relative humidity room to further dry for another four days.

Seedling counts were conducted on 24 November (85 days after sowing) within every 2 m section of row sown by the cone seeder and within each 2 m placement sown by the niche seeder.

As the strips sown by each machine were unreplicated and located 70 m apart, direct comparisons between the success of each machine were precluded. However, comparisons between priming treatments within each sowing method were conducted, using paired Student's t-tests.

Figure 37. *Atriplex nummularia* direct seeding demonstration strip sown by a commercial "Mallen" niche seeder. Approximately 25 seeds were placed every 2 m.

Results and discussion

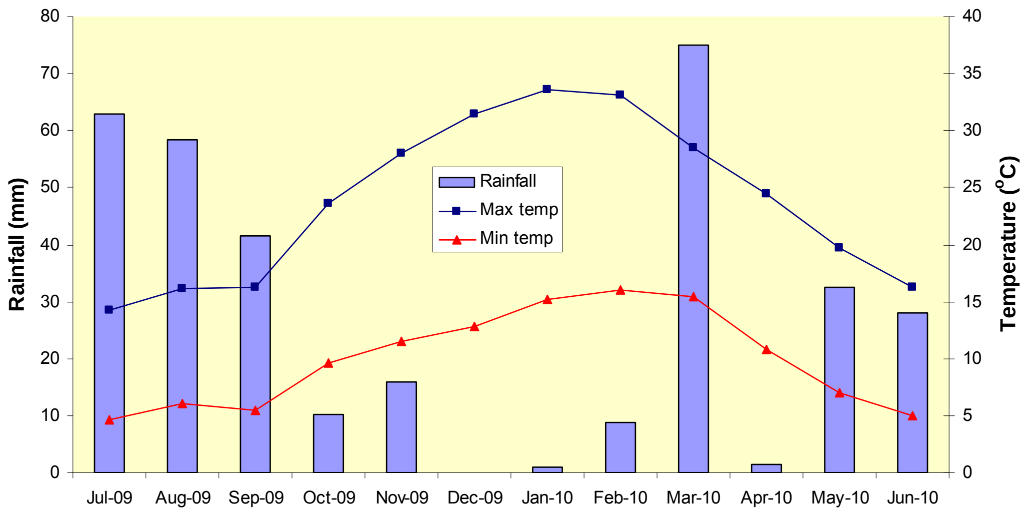


Figure 39. Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 at Wickepin, 25 km north of the demonstration site (Data from the Australian Bureau of Meteorology).

Monthly rainfall totals and mean maximum and minimum temperatures from 1 July 2009 to 30 June 2010 are shown for Wickepin in Figure 39. Germination conditions were very favourable. The soil surface was very moist at sowing and remained moist until mid-October, with 39 mm of rain falling in the five weeks after sowing (Figure 39).

Establishment was successful from both machines, with the cone seeder having an establishment rate of 17% of all seed sown and the niche seeder 15% (Table 13). Figure 40 shows the resulting establishment in the cone-seeded strip. Priming with kinetin did not affect establishment of seed sown with the cone seeder, supporting results from both Wagin and Mingenew. However, priming produced a significantly higher established density of seed sown by the niche seeder. Although valid comparisons between the establishment success of seed sown by both machines cannot be made, the establishment percentage of un-primed seeds was lower for the niche seeder, while that of primed seeds was similar between machines. This suggests that *A. nummularia* establishment using a commercial niche seeder might be improved if seeds are primed with a signalling compound.

Figure 40. Establishment of *Atriplex nummularia* at Wickepin in the demonstration strip sown by a cone-seeder on 7 September 2009. Photograph taken on 24 November 2009.

8. An establishment package for *A. nummularia*

This project has demonstrated that direct seeding of *A. nummularia* is quite feasible using conventional seeding equipment. The results of this project and from industry experience suggest the following principles for reliable establishment of *A. nummularia* through direct seeding.

1. Select appropriate paddocks

Good site selection is critical to successful establishment. The most appropriate paddocks are those with slightly- to moderately-saline land (EC_e values at 0-30 cm depth between 2 and 8 dS/m). Productivity is markedly reduced on extremely saline land (EC_e > 32 dS/m). *Atriplex nummularia* is susceptible to waterlogging, so avoid areas prone to prolonged waterlogging. Sites with 50-100% annual ryegrass and some slender ice plant are generally ideal. If the site has samphire or is bare and scalded, then it is too saline. If the site has substantial sea barleygrass, then it is too waterlogged. Duplex soils with sandy loam over clay are the easiest to establish, as the sandy surface allows salts to leach through the soil following winter rains, creating a favourable environment for germination. More details on appropriate site selection are provided in Barrett-Lennard *et al.* (2003).

2. Prepare sites for optimum establishment

A weed-free seed bed is essential, as *A. nummularia* seedlings are weak competitors. Plan a year ahead if possible and reduce weed seed-set in the year before sowing by grazing and spray-topping, especially for ice plant and annual ryegrass. Commence control of rabbits and kangaroos if they are a potential problem.

A good weed control strategy is to use two knockdown sprays (4-6 weeks and 1-2 weeks before seeding). Cultivation prior to sowing can make the surface too uneven for precise sowing. Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

3. Sow the best seed

Germination of *A. nummularia* seed batches is generally low, and typically varies from 5–20%. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. The following steps will help maximise use of high seed quality.

Ensure seed is subspecies nummularia

This project indicates that subspecies *nummularia* is best suited to direct seeding. The alternative subspecies, subsp. *spathulata*, appears to have different requirements for germination and does not readily establish from direct seeding (see Section 6). Other studies in the “Enrich” project of the FFI CRC have also shown that subsp. *nummularia* is much more palatable to grazing sheep than subsp. *spathulata*.

Ensure seed is fresh

Use seed harvested within the previous 6 months that has been stored in a cool, dry environment.

Use large heavy fruits

Large and heavy fruits should be selected for sowing, as they are more likely to contain mature, viable seeds. Small fruits, on the other hand, are more likely to be empty or contain undeveloped embryos. If there is a large range in fruit size, grade off the smaller fruits.

Retain bracts

Do not remove the bracts surrounding the fruit, as establishment success in the field has been higher for seed with bracts, compared to seed with their bracts removed (see Section 4.1).

4. Sow into moisture in late winter-spring

The ideal time for sowing is a compromise between soil temperatures being warm enough for germination and the likelihood of there still being sufficient moisture for good root development before summer. On saline land, sowing into moisture in late winter-spring also means salts are more likely to have been flushed from the soil surface. This time of year also allows good control of winter weeds and insects prior to sowing.

The ideal sowing window is earlier in more northerly districts than more southerly ones. For example, the best sowing times for different regions in Western Australia are:

- Northern agricultural region - early to late August
- Central and eastern wheatbelt - mid-August to early September
- Southern agricultural region - late August to late September

In areas with more reliable spring and summer rainfall, such as northern New South Wales, the sowing window will be more extended.

Seeds should be sown into a moist seedbed, or if there is a strong likelihood of rain.

Although *A. nummularia* is salt tolerant, its germinating seeds are susceptible to salinity and will not germinate if the soil surface is too saline. Rainfall leaches salts from the surface, providing a relatively fresh environment for germination. If the soil surface in a saline environment is dry, it is quite likely to be too saline for germination of *A. nummularia*.

If the area to be sown is waterlogged, sowing should be deferred until later in spring, or to the following year.

5. Aim to establish at least one plant per 2 m of row

Use a sowing rate of ~10 fruits/m (if germination rate is 15%). This should provide at least one plant every 2 m, allowing for losses of ~60%. Higher rates should be used for seed of lower germination.

6. Set-up the seeder for the best result

A standard Massey Ferguson combine seeder (or something similar) can be used to direct seed old man saltbush, with some minor modifications. The simplest way is to remove tynes not needed for sowing. Small boxes (one for each seeding tyne) can be attached to the seeding box to hold the small quantity of seed required, with hoses attached to seeding tynes. Seeders should also have the following features.

Formation of furrows

Tynes should create furrows to capture rainfall and increase seedling survival, particularly in dry springs. Furrow formation also scalps away non-wetting sand and removes weed seeds. Furrows should be formed to minimise soil in-fill and should be broad to increase the area of rainfall capture.

The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting, provided furrow collapse and soil in-fill is avoided, as this causes burial of the seed, which can markedly reduce seedling emergence

Press wheels

Press wheels provide good seed contact with soil moisture and reduce in-fill of soil into the furrows. They should press soil in the furrow bottoms and minimise soil in-fill from the sides. Flat-bottomed wheels give the best results.

Row width

Calculate the desired width between saltbush rows for each seeding pass and adjust the width of seeding tynes accordingly, removing non-seeding tynes. Typical row widths for alleys of double or triple rows are 1 m. A stand of saltbushes 1 m x 1 m apart can be regarded as being very dense.

7. Sow to a depth of 5–10 mm

Atriplex nummularia requires shallow seeding to a depth of 5–10 mm. Seeds sown too deep will not emerge. The simplest method is to drop fruits in the bottom of furrows and press them in with press wheels. When correctly adjusted, this will leave a small proportion of fruits visible on the soil surface. Sowing directly onto the surface is unreliable.

8. Control weeds and pests (insects, kangaroos and rabbits)

Summer growing weeds compete strongly with saltbush seedlings for soil moisture. Control weeds with appropriate herbicides to maximise establishment. Monitor the paddock for insect damage, especially over the first eight weeks and control if needed. Good control of kangaroos and rabbits is essential to protect young seedlings.

9. Defer grazing until seedlings are well established

The first grazing of *A. nummularia* bushes should be deferred until they are well established and actively growing. This will vary with seasonal conditions and may not be until after the break of the next season. Ensure plants are firmly anchored before introducing animals.

9. Establishment of other species

9.1. Rhagodia preissii

Results of Section 5.1 suggest *R. preissii* can be established using the same direct seeding methods as for *A. nummularia*. However, this is based on the success of just one trial in the northern agricultural areas of Western Australia. This is the region where *R. preissii* is being promoted, but further trials are required to demonstrate repeatability of establishment in more southerly areas, with cooler soil temperatures, and on other soil types.

9.2. Other Atriplex species

This project was not able to develop reliable establishment packages for other *Atriplex* species including *A. amnicola* and *A. undulata*. The project had limited success with these species, which presently work well with the "Mallen" niche seeder. Further work is needed to understand the triggers for seedling emergence before these species can be direct-seeded with conventional machinery.

9.3. Maireana brevifolia and *M. pyramidata*

Direct sowing of *M. brevifolia* and *M. pyramidata* appears to be problematic in much of southern Australia. The main difficulty is their requirement for warm temperatures (~30^{o}C) to germinate (see Section 3.1). These temperatures do not normally occur until November in southern Australia, by which time the winter growing season has generally ceased. This precludes the direct seeding of these species without the aid of irrigation. An exception to this would be areas with more reliable summer rainfall, such as northern New South Wales. Here, sowing could be deferred until late spring-early summer.

Field observations indicate widespread recruitment of new *M. brevifolia* seedlings from surrounding bushes, most likely after episodic summer rainfall events (P.G.H. Nichols and E.G. Barrett-Lennard, unpublished data). This suggests an alternative and potentially cheap method of establishing *M. brevifolia*, if it is already present in the area, is to encourage natural recruitment of seedlings from seed produced on surrounding bushes. However we need ways to get at least small numbers of viable plants into virgin sites. One way of doing this could be to transplant a low density of nursery-raised seedlings which could then act as a seed source for natural recruitment. It is likely *M. pyramidata* could be established in a similar way. In order to devise strategies to increase recruitment of new seedlings, there is clearly a need for studies to better understand the ecology of *M. brevifolia* and *M. pyramidata* and their cues for germination. It may also be possible to identify populations or genotypes of both species with lower temperature requirements for germination, that make them more suited to direct sowing in late winter or early spring.

10. Conclusions and further work

10.1. Seeding machinery

This project has illustrated the feasibility of establishing *A. nummularia* and (perhaps) *R. preissii* through direct seeding with conventional seeding equipment. This raises the possibility of wide-scale plantings in mildly- to moderately saline land and rangeland country. The main principles are to scalp away non-wetting sand and weeds, and sow into furrows with precise depth control (5-10 mm). Furrows are important for harvesting rainfall, especially during dry springs and in low rainfall areas with sporadic rainfall events.

While the principles have been established, it would appear that further machinery development may be required to fine tune seeding for more reliable establishment. In areas prone to waterlogging, the current system of placing seeds in furrows below the surface may not be ideal for longer-term stand survival. These may become inundated, particularly during winter in subsequent seasons, with a resulting reduction in stand survival. One advantage of the “Mallen” niche seeder is that seeds are placed on mounds above the soil surface, reducing the effects of waterlogging. In such areas machinery design may need to be modified to enable furrow formation on top of mounds.

Some machinery modifications to form broad, shallow furrows may also be beneficial for low rainfall agricultural and rangeland areas. In such areas drought stress is likely to be a common problem and the formation of such furrows would harvest more rainfall to provide moisture to emerging seedlings.

10.2. Seed quality and seeding rates

Poor seed quality is a major limiting factor for successful chenopod establishment. Several commercial seed lots we tested had very low viability levels, with a high proportion of inert matter and empty fruits. This indicates considerable room for higher seed quality within there seed industry. A greater consumer awareness of the large variability in seed quality and encouragement to conduct formal germination testing may help in lifting standards.

Some seed-related areas leading to increased likelihood of establishment success were identified in this project. The first concerns selection of larger-heavier fruits for sowing, as they are more likely to contain mature, viable seeds. Larger fruits can be selected directly off the bush, while smaller fruits can be grading off in commercially purchased batches (these are likely to have low germination in any case). Secondly, seeds should be stored post-harvest in a cool and dry environment and should ideally be used within six months of harvest.

With *A. nummularia* this project identified the greater suitability of subsp. *nummularia* than subsp. *spathulata* for direct seeding. Other work in the FFI CRC "Enrich" and "Saltbush selection" projects have demonstrated much greater palatability of subsp. *nummularia* than subsp. *spathulata*. It is concerning there is a general lack of awareness of differences between *A. nummularia* subspecies in the nursery and seed trade and among consumers. A greater distinction needs to be made between these subspecies, in order that the public buys the type that is best suited to their needs.

The identification of genotypic differences for ability to establish from direct seeding within subsp. *nummularia* means there is a possibility of breeding cultivars of *A. nummularia* with enhanced ability to establish from direct seeding. With this in mind, seed of the Emergent type, which has been identified as having a high ability to emerge from direct seeding (see Sections 3.4 and 6), has been provided to the FFI CRC old man saltbush breeding program for incorporation as a breeding objective.

The biggest limitation in determining appropriate seeding rates is seed quality. The proof of concept trials for direct seeding of *A. nummularia* (reported in Section 6) indicate a sowing rate of ~10 fruits/m is feasible to produce an established plant every 2 m. However, this is based on a germination rate of 15% and seedling losses of ~60%. Further work is needed to determine the most appropriate seeding rates, but a good understanding of the quality of the seed lot to be sown is clearly needed before this can be determined.

If seeding rates can be reduced reliably to the order of ~10 seeds per linear metre, sowing costs will be markedly reduced over the "Mallen" niche seeder, which commonly uses 50 seeds per 2 m placement, making it more attractive to sow saltbush over wide areas. Premium seed lines that are likely to be developed through the FFI CRC old man saltbush breeding program will most probably be more expensive than wild seed lots and we need to be able to get the best result with tiny amounts of seed per hectare.

10.3. Use of plant signalling chemicals to enhance germination and emergence

The most commonly used saltbush across southern Australia is presently old man saltbush (*Atriplex nummularia* subsp. *nummularia*). No case can be made based on our work for the use of plant signalling compounds to improve the establishment of this species.

Plant signalling chemicals were identified that significantly promoted germination under laboratory conditions in otherwise physiologically dormant species such as *Maireana brevifolia*, *Atriplex semibaccata* and *Rhagodia preissii*. However, this improvement was only transferred to the field for *R. preissii*. Given the inconsistency of results in the field, it is unlikely that the seed industry would adopt this technology, unless delivery of the benefits can be markedly improved.

10.4. The role of bracteoles in regulating germination

The field results in this project conflicted with the laboratory results of Stevens *et al.* (2006), who showed enhanced germination from removal of bracteoles in *A. undulata*. In *A. nummularia*, it appears that the bracteoles absorb moisture and act to regulate germination. As a result, it takes longer for bracted seeds to germinate than naked seeds. However, this appears to give an advantage under field conditions, as the bracteoles only allow the seeds to germinate under optimal conditions, hence increasing the chances of seedling survival. Further work is needed to gain a greater understanding of the role of bracteoles in regulating germination of chenopods. At present we recommend that old man saltbush be sown with the bracteoles intact.

11. Acknowledgements

This project was first conceived by Dr Mike Ewing and Dr Warren Mason and initiated as part of the former CRC for Plant-based Management of Dryland Salinity, which made a direct funding contribution. Funding was also provided by Meat & Livestock Australia, Australian Wool Innovation Ltd and Land and Water Australia. We are grateful for the interaction and support of the Saltland Pastures Association. The project team wishes to thank the following farmers and their families for use of their properties for field work: Colin Pearse (Meckering), Chris English (Wagin), Greg and Nathan Astbury (Wickepin), Ian Bagley (Mingenew), and Andrew Lee (Dumbleyung).

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Establishment of native perennial grasses

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Abstract

Native perennial grasses have a dual role to play in southern Australia. From an agricultural perspective, they are well adapted to local environmental conditions and can provide nutritious feed for livestock. They can also be used for rehabilitation of degraded land and to redress the loss of plant biodiversity. Currently, low availability of viable seed and poor seedling establishment commonly results in unsuccessful native perennial pasture production. By alleviating seed-based issues, pasture productivity will increase and land restoration with native grasses will become more attractive.

The objective of this project was to address these problems through investigation of the best means to achieve a high establishment success via alleviating after-ripening, developing mechanisms to break seed dormancy, using seed coatings and seed priming. Seed quality issues (i.e. viability, germination rates, maximum germination percentage) were assessed in 17 species across 53 ecotypes collected from QLD, VIC and WA. Based on preliminary results many of these species posses physiological dormancy, which appears to be significantly influenced by seed covering structures (their removal increases germination). Many grass seeds exhibit after-ripening phenomena, which is strongly linked to storage conditions. We assessed the effects of post-harvest storage on germination optimising temperature/humidity to maximise the rate of after-ripening.

We also investigated priming seeds with germination promoters (including smoke water, the novel chemical isolated from smoke – Karrikinolide, and gibberellic acid), removal of covering structures and seed burial to enhance germination. These experiments were undertaken in multiple dormancy breaking studies in controlled environments.

Seed priming technology was used to alleviate slow and asynchronous germination. Seed priming with the addition of stress tolerance chemicals including the plant signalling compounds salicylic acid and kinetin was used to improve establishment success of species under a range of stressful conditions that limit seed germination (i.e. drought and high salt).

Seed coating technology was applied to florets and cleaned seeds of five species. The germination enhancing chemical gibberellic acid was incorporated into the polymer pellet and film coats to determine whether seed coating can improve germination rates and percentages in the field and improve seed delivery to site.

Treatments that delivered improved vigour without detrimental effects were transferred to the field. Field trials were established at the Department of Primary Industries Walkamin Research Station, Queensland and Victoria and Department of Food and Agriculture, South Perth WA and Shenton Park Research Facility WA. At each site treatments of accessions were planted into $100m^2$ plots using a completely randomised design. Results are only presented for WA trials.

This work was conducted through the project "*Grass Roots – native perennial grasses for sustainable pasture systems*", funded by the Rural Industries Research and Development Corporation (RIRDC), with links to the Future Farm Industries Cooperative Research Centre (FFI CRC) project "*Reliable establishment of non-traditional perennial pasture species*".

Seed quality is a significant issue with native grasses, particularly if collected from wild stands. This project highlights two techniques (X-ray analysis and air separation) that provide rapid means to improve seed quality for end users.

An understanding of the species/accession specific germination biology has allowed significant improvements in germination performance of native grass seeds. The use of

dormancy breaking treatments and germination stimulants has further improved germination performance of several species of native grass seed in laboratory environments, for example:

- *Austrodanthonia* species respond well to smoke water treatment at 1% solution, which increases germination from 26% to 96% if seeds are cleaned
- *Chloris truncata* germination can be increased in some ecotypes from 5% to 48% when treated with gibberellic acid and further increased to 86% if also heated at 100°C for 30 minutes
- this heat treatment will also increase germination of *Themeda triandra* seeds from 5% to over 30%
- Gibberellic acid enhances germination of *Dichanthium sericeum* from 9% to 74% if the florets remain intact; if the caryopses are removed >90% germination can be achieved without further treatment
- *Enteropogon acicularis* germination was improved from 48% to 99% by removing the caryopses from the enclosing floret structures without the need for germination enhancing chemicals
- *Microlaena stipoides* seeds germinate to between 90 and 100 % when fresh either as intact florets or as cleaned seeds without germination stimulants but gibberellic acid may increase the rate of germination.

The use of stress signalling compounds appears to have a broader effect on native grass species, with salicylic acid and kinetin improving germination under water and salinity stress in many species. Response to drought stress was species specific however *M. stipoides* appears to be the most resilient native grass tested, at least at the germination phase.

After-ripening and storage treatments highlighted species differences. However when stored under ideal conditions (cool and dry), there appears to be no negative effect of storage in *A. caespitosa* and *M. stipoides* after 6 and 12 months respectively.

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species/accessions tested. The approach used in this report can be readily adapted to industry to improve the quality of the end product. This quality increase will hopefully facilitate broad scale adoption of native grasses in Australia.

This study highlights the capacity to manipulate native grass seed germination under stressful conditions to improve germination success. Several key seed treatments have been identified as showing promise including, (a) seed cleaning (b) seed burial for favourable water relations and (c) seed priming through the use of smoke water, gibberellic acid, salicylic acid and kinetin. The translation to field establishment requires further research despite alleviation of some barriers to germination.

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1. Introduction

Large areas of Australia's land are ecologically degraded including those that have been cleared and are now subject to erosion, rising water tables, dryland salinity, weed invasions or other ecological problems. This indicates that current farming systems (high-nutrient-requiring annual crops and pastures with shallow root systems) are not sustainable (Lodge 1994). There is an urgent need to identify perennial species that have the potential to be successful in the low to medium rainfall (<300-500 mm) regions of Australia. Commercial pasture species in southern Australia have been imported from the Mediterranean basin, the basis being that such species are well adapted to both extreme climatic conditions and can withstand grazing pressure. However, Australia with its diversity of climate and growing conditions has a very rich native biodiversity of grass species, many of which were initially overlooked for agricultural value. Given the large genetic diversity which exists, there is huge potential for the development of native perennial species that are already well adapted to the climatic/edaphic conditions of Australian farming/pasture systems (Bennett *et al*. 2003).

1.1 Native Grass Species Attributes

Previous research on developing low input grasses useful in limiting environments (LIGULE) has identified native grass species with pasture potential (Johnston *et al*. 1999). Species were chosen from a range of attributes including persistence, vigour, productivity, palatability, morphology, and characteristics related to seed production (Mitchell *et al.* 2001). The LIGULE study determined that research was needed to develop methods of producing seed cheaply and efficiently.

Currently, low availability of viable seed, asynchronous and unreliable seed germination, poor seedling emergence and low seedling vigour result in variable and unsuccessful native perennial pasture production. This has resulted in large associated costs of seed production (Crosthwaite *et al.* 1996), unreliable establishment leading to high establishment costs (Vere *et al.* 2002) and limited broad-scale adoption of native perennial grasses. By addressing these fundamentally limiting factors through investigation of the best means to achieve high establishment success via alleviating after-ripening, breaking of seed dormancy mechanisms and use of the new innovations of seed priming and coating, it is anticipated that greater establishment of native perennial grasses at lower costs will inevitably result in a more successful native pasture industry.

1.2 Target Species

Native perennial grass species have been evaluated for use in agricultural pasture systems. Seventeen species have been identified which show great promise for Australian pasture systems in either northern or southern Australia (Table 1). Target species/genera were determined prior to project commencement based on careful coordination with perceived markets, identified by Native Seeds Pty Ltd, and research criteria requirements, developed by the CRC for Plant-Based Management of Dryland Salinity.

Suitable markets now exist for native perennial pastures that have naturally developed greater suitability to problem areas such as acidic soils, saline and waterlogging susceptible soils where use efficiency is an important consideration, low input farming systems (i.e. low nutrient), and areas susceptible to pests and disease.

2. Objectives

The objective of the project was to develop a national program to deliver seed-based solutions to overcome seed germination barriers for a profitable and productive broad-acre native perennial grass pasture and restoration industry. This aim will be met via the following objectives:

- refine new advances in seed production technology to increase commercial availability of native perennial grass species (including the use of the discovery by the applicant of one chemical in smoke that stimulates germination of native species) for pasture systems and rehabilitation sites

- define and prioritise the information and implement technology required to overcome barriers to commercial production, focussing on improving seed germination, seedling vigour and seedling stress tolerance

- increase the efficiency of seed-to-site establishment, by targeting a series of high potential native pasture species and recent innovations in seed technologies including polymer-based seed coatings for efficient delivery of germination enhancement and growth promoters.

3. Materials and Methods

3.1 Species Selection

Seventeen species have been identified which show great promise for Australian pasture systems in either northern or southern Australia (Table 1). Of these 17 only nine have been available for evaluation in this project. As previously mentioned, target species/genera were determined with input from Native Seeds Pty Ltd and the CRC for Plant-Based Management of Dryland Salinity. Other species have been collected from wild populations in the Western Australian wheat-belt region, six species from Victoria and one from Queensland.

Table 1: Species used for trials, including cultivated species and wild collections. Collection locations include several populations from northern and southern Australia. Rows highlighted in grey indicate the species chosen for evaluation by Native Seeds Pty Ltd, and CRC for Plant-Based Management of Dryland Salinity.

Species	**Collecting No/ Accession**	**Collection Location**
Austrodanthonia caespitosa	Bod 12/05	Boddington (Perth Hills)
Austrodanthonia caespitosa	SC204	Northam, WA
Austrodanthonia caespitosa	SC206	Kellerberrin, WA
Austrodanthonia caespitosa	Dc1	Trangie NSW
Austrodanthonia caespitosa	Dc1 Medina	Medina, WA
Austrodanthonia fulva	DRf1	Monaro NSW
Austrodanthonia pilosa	SC219	Armadale, WA
Austrodanthonia racemosa	Dr1	Monaro NSW
Austrodanthonia richardsonii	Vic 12/05	Victoria
Austrodanthonia setacea	Meredith	Victoria
Austrodanthonia setacea	SC222	Mundaring, WA
Austrodanthonia setacea	Kowarra 7/11/07	Echuca, VIC
Austrodanthonia sp. Goomalling	SC210	Quairading, WA
Austrodanthonia "Bunderra"		Victoria
Austrodanthonia "Taranna"		Victoria
Bothriochloa macra	NS418	Tamworth, NSW
Chloris truncata	JS - York	York, WA
Chloris truncata	SC210	Quairading, WA
Chloris truncata	SC217	Grass Valley, WA
Chloris truncata	SC226 27/10/07	Miling, WA
Chloris truncata	Geraldton 04/08	Geraldton, WA
Chloris truncata	Northam 03/08	Northam, WA
Chloris truncata	NS285	Myrtleford (Native Seeds Pty Ltd)
Chloris ventricosa	NS281	Myrtleford (Native Seeds Pty Ltd)
Dichanthium sericeum	D11a	Department of Primary Industries, QLD, ecotype 11
Dichanthium sericeum	D11b	Department of Primary Industries, QLD, ecotype 11
Dichanthium sericeum	D16a	Department of Primary Industries, QLD, ecotype 16
Dichanthium sericeum	D16b	Department of Primary Industries, QLD, ecotype 16
Dichanthium sericeum	Euchla 25/02/08	Echuca, VIC
Enteropogon acicularis	Native Seeds 2005	Victoria
Enteropogon acicularis	Medina 25/02/08	Medina, WA
Heteropogon contortus	S8a	Department of Primary Industries, QLD, ecotype 8
Microlaena stipoides	Griffin (NS449)	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin (NS292) Early	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin (NS292) Late	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin A1108	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin A2108 1st	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin A2108 2nd	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin B1108	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Griffin B1208	Wangaratta (Native Seeds Pty Ltd)
Microlaena stipoides	Lig 183 (13/12/06)	Echuca, VIC
Microlaena stipoides	Lig 183 (22/01/07)	Echuca, VIC
Microlaena stipoides	Lig 183 (13/02/07)	Echuca, VIC
Microlaena stipoides	Lig 183 (23/03/07)	Echuca, VIC
Microlaena stipoides	Lig 183 (03/05/07	Echuca, VIC
Microlaena stipoides	Lig 183 (07/11/07	Echuca, VIC
Microlaena stipoides	Bremmer (NS290)	Wangaratta (Native Seeds Pty Ltd)

Microlaena stipoides	Ovens (NS289)	Wangaratta (Native Seeds Pty Ltd)
Themeda triandra	K20a	Department of Primary Industries, QLD, ecotype 20
Themeda triandra	K20b	Department of Primary Industries, QLD, ecotype 20
Themeda triandra	K24a	Department of Primary Industries, QLD, ecotype 24
Themeda triandra	K24b	Department of Primary Industries, QLD, ecotype 24
Themeda triandra	Bod 02/06	Boddington (Perth Hills)

3.2 Seed Quality and Viability Assessment

Seed viability and/or seed fill was determined on every seed batch by low intensity, high-resolution x-ray analysis (Faxitron specimen radiography system). This indicated the number of florets that contain caryopses (Fig 1). The x-ray unit is designed for high resolution radiographic imaging of medical specimens, in particular, tissue and bone. This unit has shown to be extremely successful for use in seed analysis and allows for rapid and multi-sample determination of seed viability, non-destructive auditing of seed quality, non-invasive interrogation of internal seed morphology and real-time investigation of seed dormancy, embryo maturation and growth.

(a) (b)

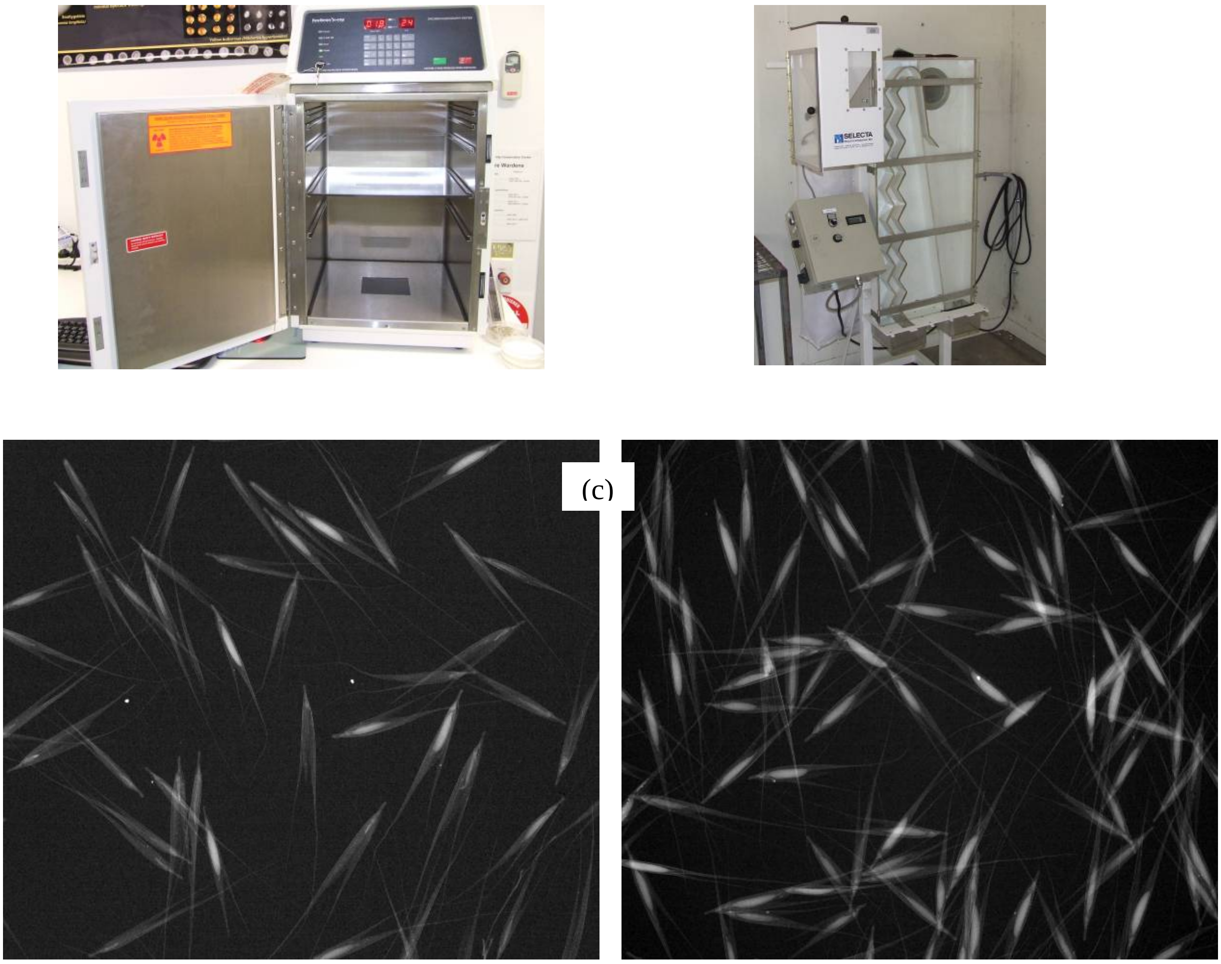


Figure 1: (a) Faxitron X-ray machine used to non-destructively identify filled florets (b) The Air separator Zigzag-1 machine used to separate filled (heavy) florets from non filled (light) florets and (c) an X-ray image of a *Microlaena stipoides* seed batch showing floret fill of the initial batch (left) and 100% floret fill (right) after separation using the Zigzag machine.

After the initial seed batch assessment, if the batch has been identified as containing empty or poor quality seed, it will be put through Air separator zigzag machinery to separate the empty (light) florets from the filled (heavy) florets, thus ensuring optimal quality and seed fill (Fig 1) for use in experimental trials.

3.3 Germination Enhancement

3.3.1 Germination stimulating treatments

All species were tested for initial germination to identify the presence of seed dormancy mechanisms and alleviation requirements. Treatments examined the impacts of surrounding structures on germination/dormancy behaviour (intact florets vs. hand clean seed), heat shock to overcome after-ripening (no heat vs. seed heated at 100°C for 30 minutes) (Tieu *et al.* 2001), application of germination stimulants including gibberellic acid (100 ppm), smoke water (1%) and karrikinolide (KAR_1) (100 ppb), all known to stimulate germination and/or alleviate physiological dormancy (Dixon *et al.* 1995, Flematti *et al.* 2004), a phenomena common to the Poaceae. For germination stimulant experiments, all seeds were soaked in treatments for 24 h before plating onto 82 mm diameter glass microfibre filter paper in 90 mm plastic Petri dishes soaked with tissue culture grade water. There were four replicates of 25 seeds per treatment. Petri dishes were sealed with parafilm and incubated at 20°C in the dark. All plates were scored for germination at 7, 14, 21 and 28 days.

3.3.2 Statistical analysis

Data was analysed using SigmaStat statistical analysis software**.** Germination stimulating treatments were analysed using ANOVA. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.4 Field Trials

3.4.1 Germination stimulating treatments

The aim of this experiment was to observe if germination enhancement of grasses obtained in the laboratory could be readily transferred to soil and field conditions. Germination treatments (as described above, section 3.3) were applied to two accessions of *M. stipoides* (Griffin and Ligule 183) and one accession of *A. caespitosa* (Dc1), *C. truncata* and *D. sericeum*. Both *M. stipoides* lines were tested with control, H_20 and GA only as previous trials indicated that these treatments were the most effective for germination. For *A. caespitosa, Chloris truncata* and *Dichanthium sericeum* seeds were tested with all priming treatments.

As field trials for C3 species were conducted in winter (average minimum temperature 7.7°C, maximum temperature 18.3°C) the laboratory trial previously tested was repeated except, all plates were incubated at 18/7°C (12h light/12h dark). As field trials for C4 species were conducted in spring (average minimum temperature 13.5°C, maximum temperature 24.2°C) the laboratory trial previously tested was repeated except, all plates were incubated at 26/13°C (12h light/12h dark). This incubation temperature was chosen as it closely replicated the temperatures experienced in the field at the time of the trial.

3.4.2 Germination and emergence trials

Glasshouse trials were conducted during autumn/winter at Kings Park and Botanic Garden. All seeds were soaked in priming treatments (as described above) for 24 h then dried for two days before sowing. Four replicates of each treatment were sown into punnets filled with silica sand (30 seeds per punnet). Punnets were watered daily and emergence was monitored every 3 days for 14 days, then at 21 and 28 days.

Field trials were established concurrently with laboratory and glasshouse trials at the Department of Agriculture and Food Western Australia (DAFWA), Perth Western Australia. The site was characterised by a sandy-loam soil type, with irrigation occurring every other day. Seed enhancement treatments were the same as above, i.e. primed for 24 h and dried back to initial water contents over two days. Each treatment consisted of 50 seeds planted in a row of 40 cm, and was replicated 4 times (Fig 2). A template press was used to accurately mark out replicate lines and bury seeds to exactly 1cm (Fig 2b, c). Emergence was monitored every 3 days for 14 days, then at 21 and 28 days. Fresh seeds of three accessions of *Chloris truncata* and two accessions of *Austrodanthonia caespitosa* were collected from the wheatbelt area of Western Australia and used for the initial germination trials. Also, two accessions of *Microlaena stipoides* from Victoria (Griffin and Ligule 183 lines), four accessions of *Dichanthium sericeum* and four accessions of *Themeda triandra* from Walkamin, Queensland and one accession of *Austrodanthonia caespitosa* (Dc1 line) from Victoria were used. The C3 trial was established in winter and the C4 trial was established in spring (see 3.4.1). Field trials in Victoria are not presented due to the unavoidable compromisation of these trials brought about by restrictions in water allocations and the drought experienced over the duration of this project.

3.4.3 Statistical analysis

Data was analysed using SigmaStat statistical analysis software**.** Germination stimulating treatments were analysed using a 2-way ANOVA design, and the glasshouse and field trials were analysed using a 3-way ANOVA design. Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

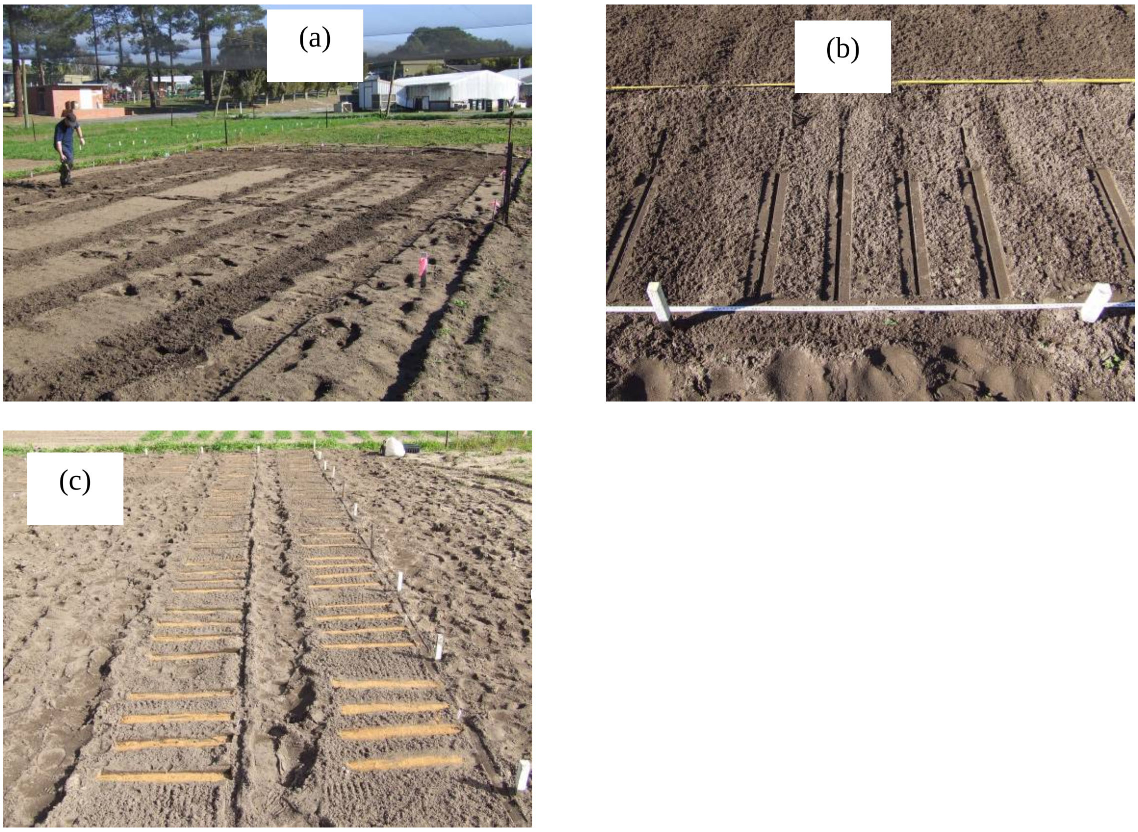


Figure 2: (a) Field site preparation (b) replicate preparation, showing burial treatment and spacing and (c) Field site after sowing at DAFWA, South Perth WA.

3.5 After-ripening

3.5.1 After-ripening treatments

Freshly harvested native grass seeds usually have at least some level of dormancy, which is released over time, known as after-ripening (Hagon 1976). After-ripening is a period of usually several months of dry storage at room temperature of freshly harvested, mature seeds and is a common method used to release dormancy and to promote germination (Bewley and Black, 1992).

In this experiment fresh seeds of *Austrodanthonia setacea, Chloris truncata, Dichanthium sericeum* and *Themeda triandra* were placed into accelerated after-ripening conditions (50% relative humidity, 45°C) to establish whether these species have after-ripening requirements for successful germination. 50% RH was chosen to imitate ambient humidity. Due to a lack of seeds available for experimental purposes it was only possible to test to test one RH% and one temperature.

Intact florets were placed into an environment of 50% relative humidity at 20°C to allow the seeds to equilibrate for 4 weeks. Seeds were then double sealed in foil bags and placed at 45°C (for the duration of the experiment).

Seeds were removed from the after-ripening environment at 1, 2 and 3 months and primed in either water (control), gibberellic acid (100 ppm) or smoke water (1%) for 24 hours. After priming, 25 seeds were placed onto glass filter papers moistened with tissue culture grade water in Petri dishes in replicates of four, except *Themeda triandra* where three replicates of 20 seeds were used due to a shortage of seeds. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 26/13°C (12 hours light and 12 hours dark). Germination was scored every 7 days for 4 weeks.

3.5.2 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software**.** Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.6 Seed Storage – the effect of temperature and relative humidity

3.6.1 Relative Humidity

Storage trials were carried out over 12 months on seed lots with two different moisture contents. Seeds were placed in relative humidity cabinets at either 20% (dry conditions) or 50% (ambient conditions) humidity using non-saturated lithium chloride solutions.

Lithium chloride (LiCl) solutions were prepared by adding 640g of LiCl to 1 L of water for the 20% RH and 370g LiCl to 1 L of water for the 50% RH. Solutions were prepared in plastic electrical boxes, tightly sealed and allowed to equilibrate for 24 hours at constant 20°C before adding the seeds.

3.6.2 Storage Temperatures

To investigate the influence of the storage environment on seed longevity, seeds were stored at three temperatures (23°C, 5°C and -18°C) and two seed water contents (20% and 50% relative humidity) as prepared above.

After equilibration for four weeks, seeds were removed from the humidity cabinets, double sealed in foil bags and placed in 23°C (room temperature conditions), 5°C (cold storage conditions) and -18°C (freezer conditions). Seeds were then removed at intervals of 3, 6 and 12 months to determine germination potential in order to monitor seed quality over time.

After storage, 25 randomly selected seeds were placed onto glass filter papers moistened with tissue culture grade water in Petri dishes in replicates of four. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.

3.6.3 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software**.** Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.7 Stress Tolerance

3.7.1 Water stress – germination at different water potentials

The effect of water potential on *A. setacea, A. caespitosa* (Dc1), *B. macra, C. truncata, E. acicularis* and *M. stipoides* (Lig 183) was investigated by supplementing germination media with either polyethylene glycol (PEG_{8000}) or sodium chloride (NaCl). PEG_{8000} and NaCl are osmolytes that are commonly used to control water potential in seed germination studies (i.e. controls the ability of seed to take up moisture). Seeds can be tested over a range of osmotic potentials (where 0 equates to soil field capacity and a more *negative* values (i.e. -1.5 MPa) equates to “drier” conditions). Osmolytes were added to germination paper equating to iso-osmotic potentials of 0, -0.25, -0.5, -0.75, -1 or -1.5 MPa. The osmotic potentials of PEG_{8000} or NaCl were calculated by equations 1 and 2, respectively, where x is the concentration of PEG_{8000} by %w/v (Michel and Kaufmann 1973) or the concentration of NaCl (mM).

$$\psi_{PEG}\ (\mathrm{MPa}) = -7.6049x^2 - 33.025x + 4.83 \qquad (1)$$

$$\psi_{NaCl}\ (\mathrm{MPa}) = -0.0045x - 0.0218\ (R^2 = 0.9981) \qquad (2)$$

3.7.2 Germination under saline conditions

To improve germination vigour of *A. setacea*, *A. caespitosa* (Dc1), *C. truncata* and *M. stipoides* (Lig 183) under three saline conditions (0, -0.5, or -1 MPa NaCl), the plant signalling compounds: 100ppm gibberellic acid, 0.05mM kinetin (K) (Khan *et al.* 2003), and 0.5mM salicylic acid (SA) (Senaratna *et al.* 2003) were examined. Salicylic acid was dissolved in 1 mL of 100% ethanol prior to adding to 1L of water (Williams *et al.* 2003). Four replicates of 25 seeds with florets removed were placed on germination papers moistened with one of the plant signalling compound/NaCl combinations. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.

3.7.3 Germination under water stress using plant signalling compounds

Treatments that resulted in the most significant improvement in germination under NaCl-induced water stress identified above were subsequently assessed as potential seed-priming agents. 100 ppm GA3, 0.05mM K, 0.005mM SA, GA3 + K, GA + SA, K + SA, GA + K + SA combinations were used to test germination of *A. setacea, A. caespitosa* (Dc1), *C. truncata, E. acicularis* and *M. stipoides* (Lig 183) under water stress (PEG_{8000} solutions with iso-osmotic potentials of 0 and -0.5 MPa). Four replicates of 25 seeds with florets removed were soaked in the seed-priming agents for 18 hours prior to plating on germination papers soaked in the PEG_{8000} solutions. Petri dishes were sealed with plastic cling wrap and placed into an incubation chamber at 20°C for 12 hours light and 12 hours dark. Germination was scored every 7 days for 4 weeks.

3.7.4 Statistical analysis

Data was analysed using a 2-way ANOVA design with SigmaStat statistical analysis software**.** Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

3.8 Seed coating technology

3.8.1 Seed coating methodology

Improved seed coating technology has been used in recent years in a range of industries, including agriculture, horticulture, turf production and floriculture. It has the ability to impact on crop yield, water use efficiency, improved germination synchronisation, final germination percentages and plant health and disease resistance.

Pellet coating is used to create a smooth, uniformly shaped pellet to improve seed flow through machinery and enable accurate delivery of seed to site. Pellets are particularly useful in fluffy grass florets where coating is applied to smooth the floret, prevent clumping and apply germination enhancing chemicals, fungicides and/or identification colourants. Film-coating is used to apply the same additives as pellets in a light film without changing the shape of the seed or floret. The pellet or film coat is produced by mixing a binder (polymer), colour, water and desired chemical (if necessary) and applying this to either the florets or cleaned seeds through specially designed machinery.

In this project we coated intact florets and cleaned seeds of *A. caespitosa* (Bod 12/05), *A. caespitosa* (Dc1), *C. truncata*, *D. sericeum* and *M. stipoides* (Lig 183) with either pellet- or film-coats. Water or GA_3 (1000ppm) were added to the coats to determine whether seed coat delivery of GA_3 improves germination synchronisation and percentages. Film coating was applied to intact florets and cleaned seeds while pellets coating (Fig 3) was applied to cleaned seeds only due to a lack of available seeds (a 50g minimum requirement for intact florets). Coating was provided by Seed Solutions Pty Ltd.

Seed coats were tested under laboratory, glasshouse and field conditions as per section 3.4.

3.8.2 Statistical analysis

Data was analysed using ANOVA with SigmaStat statistical analysis software**.** Percentage values were arc-sine transformed prior to analysis. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

Figure 3: An example of pellet seed coating technology used for native grasses.

4. Results

4.1 Germination Enhancement – laboratory conditions

The germination response of species to seed cleaning, heat and germination enhancement treatments varied within and among species, with overall germination ranging from 0 to 100% (Table 2). Thirty-eight ecotypes showed significant responses to various germination enhancement treatments with gibberellic acid being the most influential. Overall 39 ecotypes had germination greater than 80% with a best bet germination treatment and only 12 had less than 80% final germination.

Germination was significantly improved in at least one of the germination treatments tested in four out of the five major grass species including *A. caespitosa*, *C. truncata*, *D. sericeum* and *T. triandra.* Removing *A. caespitosa* caryopses from florets improved germination performance (greater than 30% observed across all accessions tested). Heat treating seeds of *A. caespitosa* significantly decreased final germination percentage and was evident across all accessions tested ($P<0.001$). Subsequent treatment of *A. caespitosa* seeds with germination stimulants either had no effect (Dc1) ($P=0.404$) or significantly improved (WA collections) germination. For WA accessions, germination was significantly higher when clean seeds were treated with smoke water ($P=<0.001$) with an improvement of up to 96% being observed (Table 2).

Cleaning seeds of *C. truncata* significantly improved germination ($P<0.001$). Heat treating seeds also improved germination up to 31% ($P<0.001$). A significant interaction between gibberellic acid and heat was detected ($P=0.009$) improving germination to 86% (Table 2). *Dichanthium sericeum* germination was significantly higher when seeds were cleaned ($P<0.001$) and/or treated with gibberellic acid ($P=0.006$) compared to other treatments. Germination was improved up to 99% compared to the control (intact, not heat treated and germinated in water). Heat had no significant effect on germination performance of *D. sericeum* ($P=0.641$) (Table 2). *Themeda triandra* germination was poor in all treatments but higher in heat treated seeds (20-30%) compared to the non-heated seeds (<10%) ($P<0.001$). There was not a significant difference between stimulating treatments ($P=0.134$) or significant interactions between heat and treatment ($P=0.890$) but germination was between 2-10% higher in the smoke water treatment (Table 2). Germination of intact florets of both *M. stipoides* accessions was initially very high (>90%) with no significant improvement being made with seed cleaning ($P>0.05$). GA improved germination from 85% to 98% in intact florets (Table 2). An interesting trend was that the C4 grasses (*B. macra*, four ecotypes of *C. truncata* and *T. triandra*) all benefited from heat treatment (Table 2).

Table 2: Successful germination stimulating treatment results showing species tested, collection number/accession, seed age (A=aged >8 weeks, F=fresh<8 weeks), initial germination (%) without treatment, highest germination (%) achieved with corresponding treatments, floret (- = minus floret; + = floret intact), heat (- =no heat; +=heat 100°C 30 mins), stimulant (H_20 = water; GA = gibberellic acid 100ppm; SW = smoke water 1%; Karrikinolide 100 ppb). Grey shading of rows indicates the species selected for evaluation.

Species	Collecting No/ Accession	Seed Age	Initial %G	Highest %G	Floret	Heat	Stimulant
Austrodanthonia caespitosa	Bod 12/05	A	8	85	CL	-	GA
Austrodanthonia caespitosa	SC204	F	1	69	CL	-	SW
Austrodanthonia caespitosa	SC206	F	32	96	CL	-	SW
Austrodanthonia caespitosa	Dc1	A	59	95	CL	-	H20,GA,SW,KAR
Austrodanthonia caespitosa	Dc1 Medina	F	42	94	CL	-	H20
Austrodanthonia fulva	DRf1	A	37	42	CL	-	KAR
Austrodanthonia pilosa	SC219	F	1	55	CL	-	SW
Austrodanthonia racemosa	Dr1	A	62	62	CL	-	H2O
Austrodanthonia richardsonii	Vic 12/05	A	70	100	CL	-	GA, KAR
Austrodanthonia setacea	Meredith	A	18	27	CL	-	GA,KAR
Austrodanthonia setacea	SC222	F	61	99	CL	-	GA
Austrodanthonia setacea	Kowarra 7/11/07	F	23	91	CL	-	GA, SW
Austrodanthonia sp. Goomalling	SC210	F	0	49	CL	-	H2O
Austrodanthonia "Bunderra"		A	88	88	CL	-	H2O
Austrodanthonia "Taranna"		A	92	92	CL	-	H2O
Bothriochloa macra	NS418	F	50	81	CL	+	GA
Chloris truncata	JS - York	F	0	63	CL	+	GA
Chloris truncata	SC210	F	0	86	CL	+	GA
Chloris truncata	SC217	F	0	90	IN	+	GA
Chloris truncata	SC226 27/10/07	F	15	92	CL	-	H20,GA
Chloris truncata	Geraldton 04/08	F	15	100	CL/IN	-	GA,SW
Chloris truncata	Northam 03/08	F	50	98	CL	±	H20,GA,SW,KAR
Chloris truncata	NS285	F	88	95	CL	-	GA/SW
Chloris ventricosa	NS281	F	30	99	CL	-	KAR
Dichanthium sericeum	D11a	F	9	95	CL	-	GA
Dichanthium sericeum	D11b	F	30	95	CL	-	GA
Dichanthium sericeum	D16a	F	65	99	CL	-	H2O
Dichanthium sericeum	D16b	F	49	97	CL	-	H2O
Dichanthium sericeum	Euchla 25/02/08	F	32	62	CL	-	GA
Enteropogon acicularis	Native Seeds 2005	A	48	99	CL	-	H20,GA,SW,KAR
Enteropogon acicularis	Medina 25/02/08	F	91	100	CL	-	H20,GA,SW
Heteropogon contortus	S8a	F	5	24	IN	-	GA
Microlaena stipoides	Griffin (NS449)	F	85	100	CL	-	GA
Microlaena stipoides	Griffin (NS292) Early	F	83	83	CL	-	H20
Microlaena stipoides	Griffin (NS292) Late	F	83	83	CL	-	H20
Microlaena stipoides	Griffin A1108	F	93	94	CL	-	SW
Microlaena stipoides	Griffin A2108 1st	F	89	94	CL	-	KAR
Microlaena stipoides	Griffin A2108 2nd	F	93	95	CL	-	SW
Microlaena stipoides	Griffin B1108	F	88	99	CL	-	KAR
Microlaena stipoides	Griffin B1208	F	92	96	CL	-	SW
Microlaena stipoides	Lig 183 (13/12/06)	F	96	96	IN	-	H2O
Microlaena stipoides	Lig 183 (22/01/07)	F	99	99	IN	-	H2O
Microlaena stipoides	Lig 183 (13/02/07)	F	53	90	CL	-	H2O
Microlaena stipoides	Lig 183 (23/03/07)	F	84	94	IN	-	H2O
Microlaena stipoides	Lig 183 (03/05/07	F	39	91	CL	-	H2O
Microlaena stipoides	Lig 183 (07/11/07	F	88	88	CL	-	H2O
Microlaena stipoides	Bremmer (NS290)	F	86	86	CL	-	H20
Microlaena stipoides	Ovens (NS289)	F	90	90	CL	-	H20
Themeda triandra	K20a	F	0	29	IN	+	H2O
Themeda triandra	K20b	F	0	24	IN	+	GA
Themeda triandra	K24a	F	0	28	IN	+	SW
Themeda triandra	K24b	F	8	39	IN	+	SW
Themeda triandra	Bod 02/06	F	27	55	IN	+	GA

4.2 Germination Enhancement – from laboratory to field

4.2.1 C3 Trial

A. caespitosa germination under laboratory conditions was significantly higher ($P<0.001$) in cleaned seeds (≥90%) compared to intact florets (30-60%) after 14 days. Priming treatment had no significant influence on seed germination ($P=0.610$). For *M. stipoides*, laboratory trials showed that removing caryopsis from intact florets significantly improved germination in both Griffin and Ligule 183 lines with germination improvement up to 20% and 60% respectively. Priming treatment had no significant influence on seed germination ($P>0.05$).

A significant improvement in seedling emergence from soil was made for *A. caespitosa* (Fig 5a) and both accessions of *M. stipoides* (Fig 5b, c). Under glasshouse conditions (Fig 4) the rate of *A. caespitosa* germination was increased up to $5\%d^{-1}$ by cleaning seed and priming with either GA or KAR_1 compared to the control (intact, surface, not primed). Burying seed appeared to negate the positive effect of seed priming on germination rate. The increase in germination rate of clean surface sown *A. caespitosa* seeds was accompanied by a high final percentage emergence, with 80% final germination in the GA treated seed compared to the control of 60%.

Under glasshouse conditions the rate of both accessions of *M. stipoides* was increased in surface sown seeds (Fig 5). Gibberellic acid further improved the germination rate of intact and clean seeds on the surface. Similarly, final germination was increased by 10% in *M. stipoides* (Lig 183). Buried seed germination varied between treatments in both *M. stipoides* accessions with water primed seed germination rate and final percentage higher in Ligule 183 compared to other treatments (Fig 5c).

Significant improvements were also observed in emergence of target species under field conditions (Fig 6). Overall buried seeds performed significantly better across all species, with surface sown seeds having a lower final germination percentage and a slower germination rate (Fig 5). In buried seeds, priming in any treatment (i.e. H20, GA, SW, KAR_1) further improved *A. caespitosa* germination up to $5\%d^{-1}$. However, the best germination treatment appeared to be GA as this had the highest accompanying final percentage emergence of 54% (Fig 5a) compared to the control of 30%.

Germination and emergence of *M. stipoides* under field conditions was improved with gibberellic acid priming in the Griffin line with final germination improving by (>20%) (Fig 5b). Ligule 183 showed germination of 40-60% can be achieved by cleaning seed and burying at 1cm without applying stimulating treatments. In intact buried seeds, priming with H20 or GA can improve germination by 20% (Fig 5c).

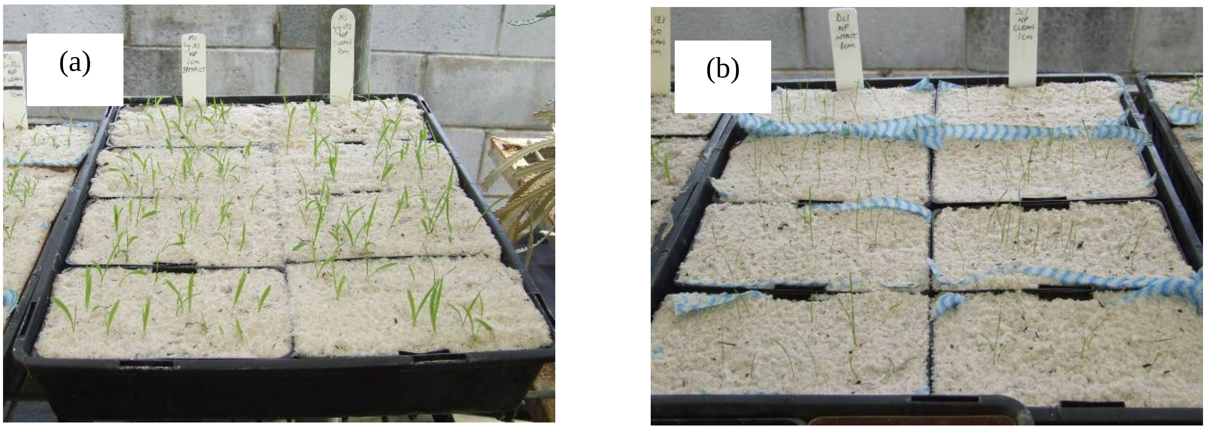
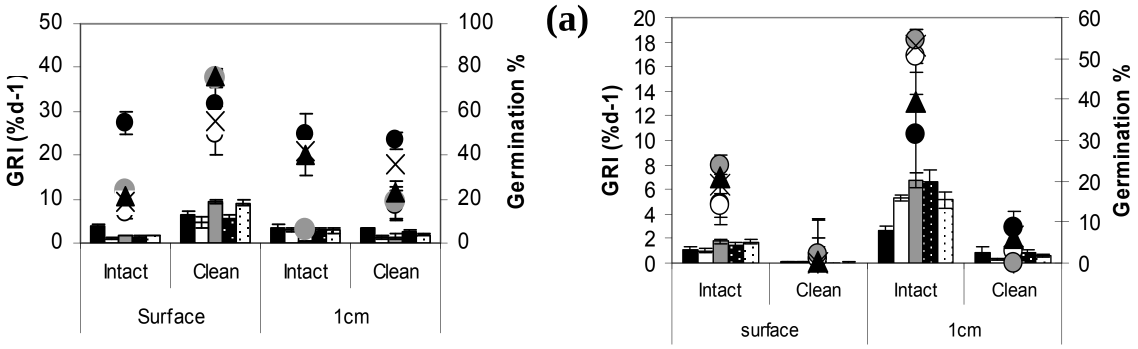
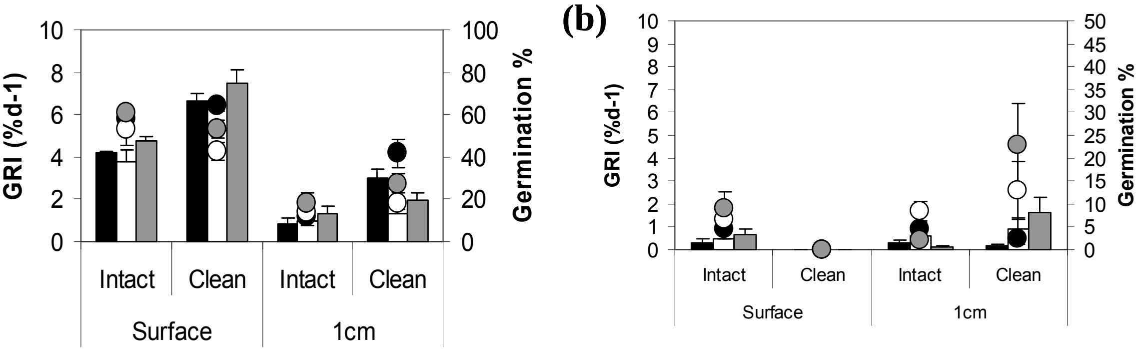


Figure 4: Photo showing experimental approach to test seed treatments under glasshouse conditions. (a) showing *M. stipoides* germination of intact seed (left) and cleaned seed (right) emerging from 1 cm depth (b) showing *A. caespitosa* germination of intact seed (left) and cleaned seed (right) emerging from 1 cm depth.

(a)



(b)



(c)

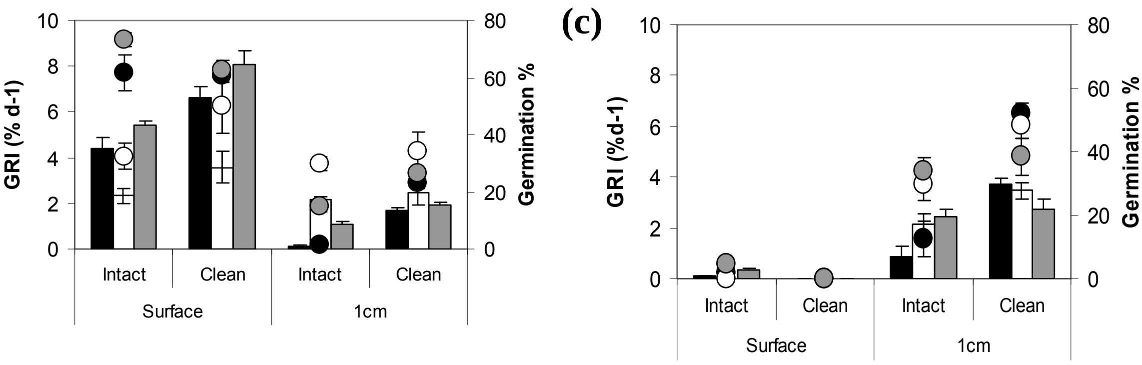


Figure 5: Germination Rate Index (GRI) and Germination (%) results for glasshouse (left hand graphs) and field (right hand graphs) trials. (a) = *Austrodanthonia caespitosa* (Dc1), (b) = *Microlaena stipoides* (Griffin), (c) = *M. stipoides* (Ligule 183). Surface = surface sown, 1cm = seeds/florets sown at 1cm depth. Intact = florets intact, Clean = caryopses removed from florets. GRI indicated by bar columns and germination % indicated by scatter plots; black = not primed, white = water primed, grey = gibberellic acid primed, black with white dots (or x for germination) = smoke water primed, white with black dots (or triangle for germination) = karrikinolide primed.

Although not part of the experiment, observations made after the summer period following the field trial highlighted that plants of *C. truncata* and *D. sericeum* derived from the GA treatment persisted to a much higher level than non-GA treated seeds (Fig 8).

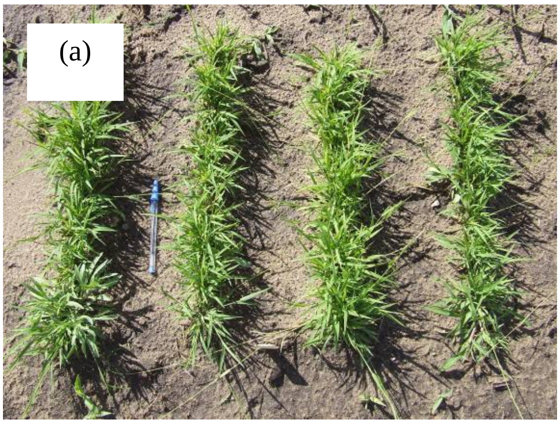


Figure 6: Photos of the field trial highlighting (a) *M. stipoides* (Lig 183) (b) *A. caespitosa* (Dc1) and (c) *M. stipoides* (Lig 183) & *M. stipoides* (Griffin) 4 months after sowing.

4.2.2 C4 Trial

Chloris truncata germination under laboratory conditions was significantly higher ($P<0.001$) in cleaned seeds (>90%) compared to intact florets ($\leq$15%) after 14 days. Heat treated seeds showed significantly less germination ($P=0.036$) compared to unheated seeds. Priming treatments had no significant influence on seed germination ($P=0.993$).

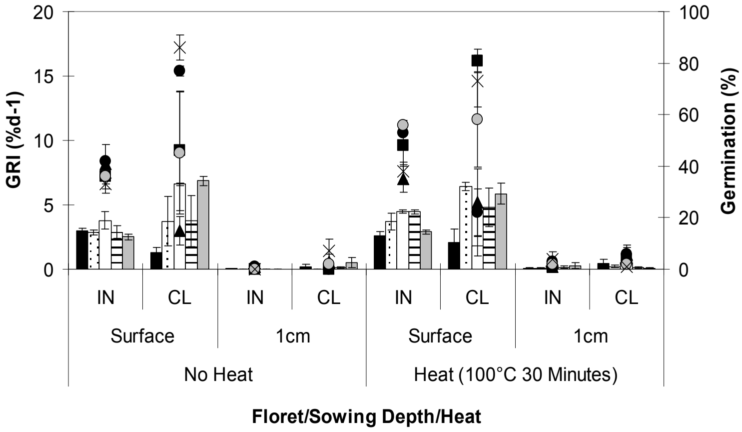
For *Dichanthium sericeum*, laboratory trials showed that removing caryopsis from intact florets did not improve germination ($P=0.575$). Similarly, heating seeds did not show a significant improvement in germination when compared to non-heated seeds ($P=0.271$). Germination was higher in water primed seeds compared to KAR_1 ($P<0.001$) and smoke water ($P=0.10$) treated seeds and higher in gibberellic acid primed seeds compared to karrikinolide ($P<0.001$).

A significant improvement in seedling emergence from soil was made for *C. truncata* (Fig 7). Under glasshouse conditions the rate of *C. truncata* germination was increased 20-fold when surface sown compared to 1 cm sown. Cleaning seed improved germination rate across all treatments (P=0.008), as did, priming with either GA or KAR_1 when compared to the control (intact, surface, not primed) (P=0.005; P=0.028). Heating seeds prior to burial did not improve germination, nor was it detrimental (0.414). The increase in germination rate of surface sown *C. truncata* seeds was accompanied by a high final percentage emergence, with an improvement of up to 40% (Fig 7, 1A).

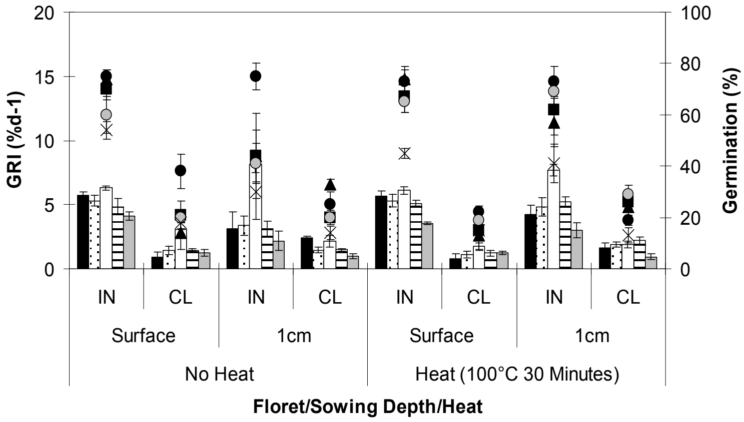
Sowing depth did not influence germination or emergence rates in *C. truncata* (P=0.166) under field conditions. Germination rate and final percentage was significantly lower in intact florets compared to clean seeds (P<0.001) when the seeds were heat treated. Priming seeds with gibberellic acid improved germination percentage (P<0.001) under certain situations compared to other treatments (P<0.001) however the best treatment was priming cleaned heat treated seeds in KAR_1 prior to burial (~10% emergence increase and 2 fold germination rate increase). Heating seeds did not improve germination percentage (P=0.733) or speed of germination (P=0.688) (Fig 7, 2A). Overall field emergence was significantly lower than under glasshouse conditions (P<0.05).

Significant improvements were also observed in emergence of *D. sericeum* under glasshouse conditions. Intact seeds germinated faster and to a higher level than cleaned seeds (P<0.001). Germination rate was further improved with gibberellic acid however this was not consistent across treatments (Fig 7, 1B). Gibberellic acid was consistently the best treatment for final germination percentage across all treatments, with up to 80% germination being observed. Heat treating seeds increased the speed of germination (P=0.009) with further increases after KAR_1 priming (P=0.036) (Fig 7, 1B). Surface sowing or heat treating *D. sericeum* seeds had no effect on germination rate index and final percentage emergence (Fig 7, 1B).

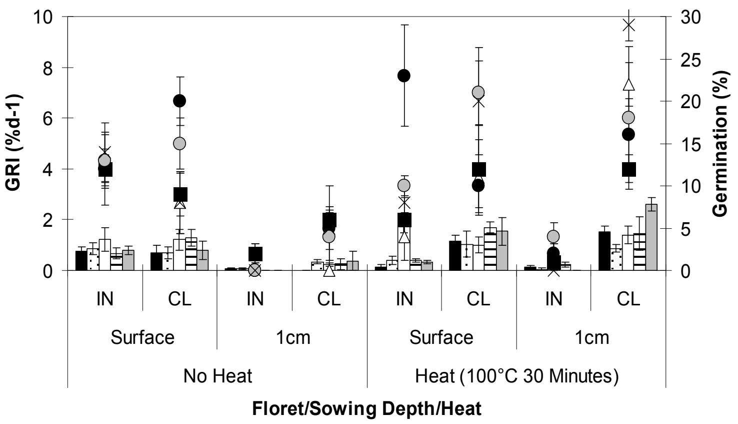
Germination and emergence of *D. sericeum* under field conditions was improved when seeds remained in florets (P<0.001) and treated with gibberellic acid (P<0.001) with germination between 10 to 15% higher compared to all other treatments (Fig 5, 2B). Seeds treated with gibberellic acid also showed a germination rate 5 to 12 times higher in intact florets sown at 1cm depth (Fig 9b). Heat treating seeds did not significantly improve germination rate (P=0.709) or germination percentage (P=0.891). The highest germination percentage was observed in non heat treated GA primed intact florets sown at 1cm depth (~70%) (Fig 7, 2B) compared to 80% in the glasshouse (Fig 7, 1B).


GRI (%d-1)
Germination (%)
IN
CL
Surface
1cm
No Heat
Heat (100°C 30 Minutes)
Floret/Sowing Depth/Heat

(1A)


GRI (%d-1)
Germination (%)
IN
CL
Surface
1cm
No Heat
Heat (100°C 30 Minutes)
Floret/Sowing Depth/Heat

(1B)


GRI (%d-1)
Germination (%)
IN
CL
Surface
1cm
No Heat
Heat (100°C 30 Minutes)
Floret/Sowing Depth/Heat

(2A)

Figure 7: Germination Rate Index (GRI) and Germination (%) results for glasshouse (1) and field (2). (A) = *Chloris truncata*, (B) = *Dichanthium sericeum*. Surface = surface sown, 1cm = seeds/florets sown at 1cm depth. IN = florets intact, CL = caryopses removed from florets. GRI indicated by bar columns; black = not primed, white with black dots = water primed, white = gibberellic acid 100ppm primed, white with black stripes = smoke water 1% primed, grey = karrikinolide 100 ppb primed. Germination % indicated by scatter plots; triangle = not primed, square = water primed, black dot = gibberellic acid 100 ppm primed, grey dot = smoke water 1% primed, X = karrikinolide primed.

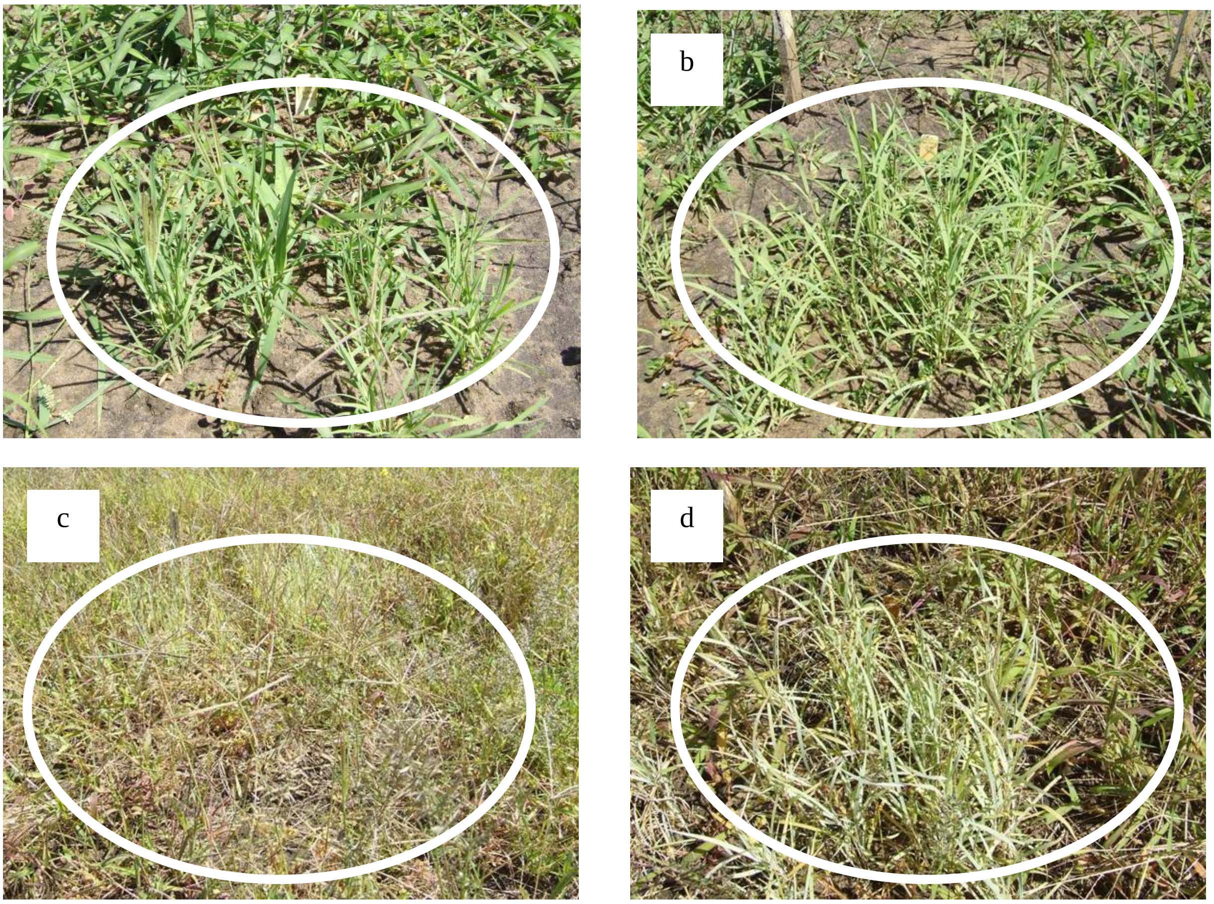


Figure 8: Results from field trial highlighting presence of GA treated (a) *Chloris truncata* and (b) *Dichanthium sericeum* 2 months after sowing (c) *Chloris truncata* and (d) *Dichanthium sericeum* 4 months after sowing. Non GA treated seeds failed to persist.

4.3 After-ripening

All four species were at least partially dormant at the beginning of the experiment (<60% germination) (Fig 9). Fresh seeds of *A. setacea* germinated to 53% with a significant increase to 71% when primed with gibberellic acid (P=0.006). After one month in after-ripening conditions, germination was slightly decreased in the control (water) and gibberellic acid treatments but significantly higher (at 75%) when treated with smoke water (P=0.029). Germination after two months was substantially less compared to the previous months with the water treated seeds only germinating up to 7%, and 24% and 19% for gibberellic acid and smoke water, respectively. Germination after three months after-ripening was zero (Fig 9a).

Dormancy of *C. truncata* seemed to be overcome after one and two months after-ripening where germination was significantly higher compared to time zero (P<0.001). After three months, however, germination was less than 1% (P<0.001) (Fig 9b). Smoke water consistently improved germination in the initial stages of after ripening. Poor initial germination of 27-33% in *D. sericeum* was not improved by after-ripening seeds or treating with germination enhancement treatments (P=0.081). The total germination percentage was reduced after one and two months with no germination occurring after three months (Fig 9c). Germination of *T. triandra* was less than 8% across all after-ripening periods and treatments, with no germination occurring after three months (Fig 9d).

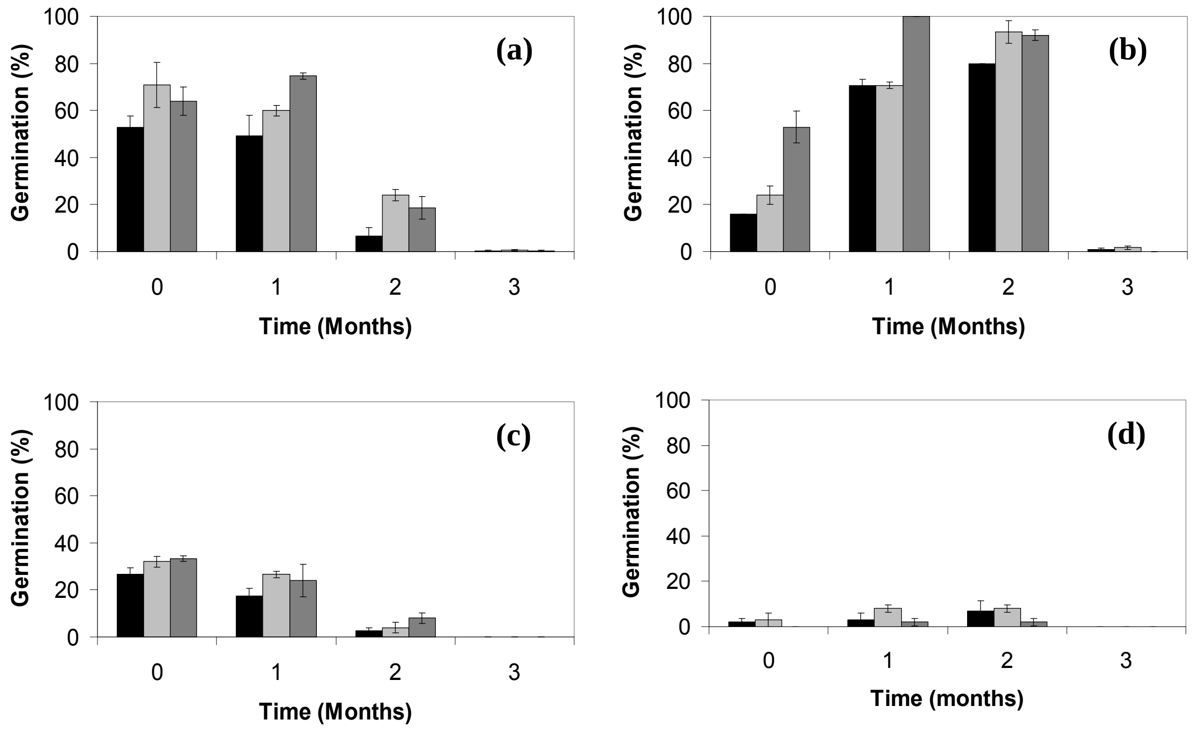


Figure 9: Seed germination percentage as influenced by 0, 1, 2, 3 months after-ripening period. (a) = *Austrodanthonia setacea*, (b) = *Chloris truncata,* (c) = *Dichanthium sericeum,* (d) = *Themeda triandra.* Germination % indicated by bar column; black = water only, light grey = gibberellic acid 100ppm treatment, dark grey = smoke water 1% treatment.

4.4 Seed Storage – the effect of temperature and relative humidity

Storage of *A. caespitosa* (Dc1) seeds was not detrimental to successful germination after six months storage at 20% and 50% relative humidity (P=0.096). Intact germination was significantly lower than cleaned seeds (P<0.001) across all treatments. However, intact seeds appeared to benefit from storage at 50%, -18°C and 5°C and germination improved when compared to storage at three months at ambient (23°C) (P=0.038). After 6 months storage, clean seeds stored in dry conditions (20% RH) showed a significant decline in germination compared to storage results at three months (P=0.020) (Fig 10a).

Germination of *M. stipoides* remained high (>80%) over the 12 month storage period in both intact florets and cleaned seeds. There was no difference in final germination percentages in the type of floret (P=0.106), relative humidity (P=0.094) or storage temperature (P=0.121). Germination was reduced, however, in cleaned seeds after 12 months storage at 23°C compared to the other storage temperatures (P<0.001) (Fig 10b). This may indicate that the viability of cleaned seeds may decline faster at warmer temperatures over time.

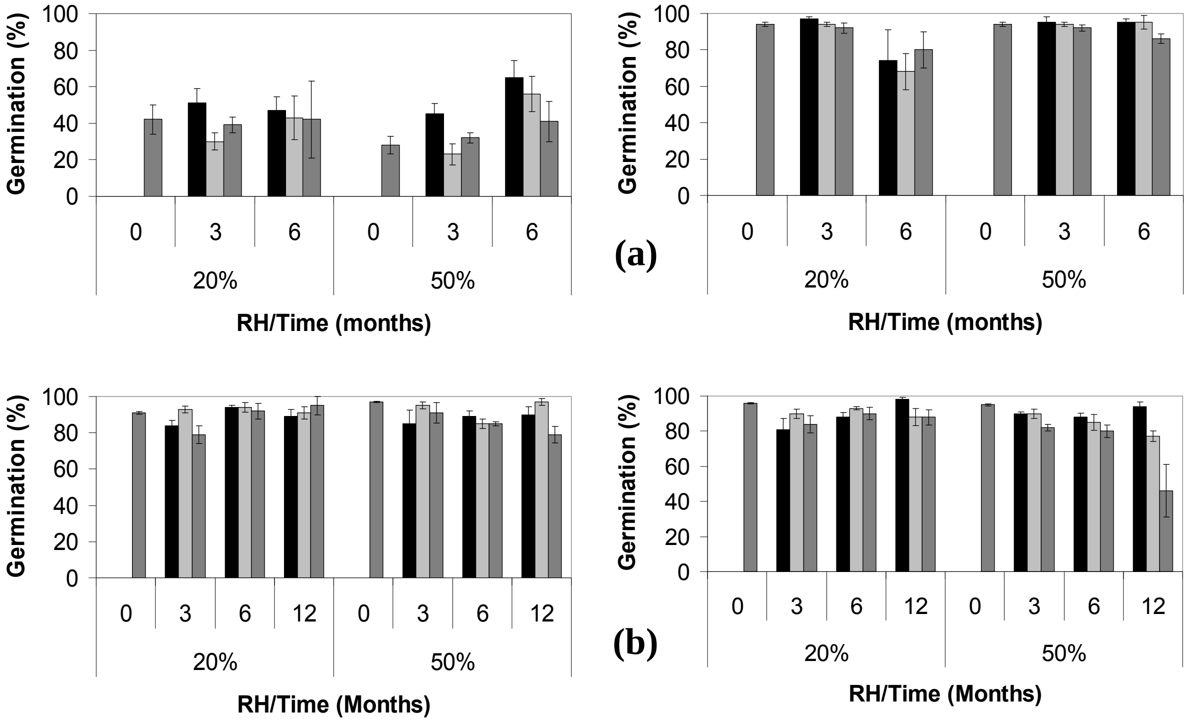


Figure 10: Germination (%) after 0, 3, 6 months storage in (a) *Austrodanthonia caespitosa* (Dc1) and after (b) *Microlaena stipoides* (Lig 183). Treatments include; intact (florets intact – left) and clean (caryopses removed from florets – right); equilibration at 20% and 50% relative humidity before storage. Germination % at different storage temperatures indicated by bar columns; black = -18°C, light grey = 5°C, dark grey = 23°C.

4.5 Stress Tolerance – germination at different water potentials

4.5.1 Water Stress

Cleaned *A. setacea* seeds have the ability to take up water more efficiently than seeds that remain in florets (Fig 11a). Germination of seeds remaining within the floret was up to 38% when water was not limited (control), but when seeds were removed from the florets (clean) germination reached 90% in control seeds and were still able to germinate in moderately dry (i.e. -0.75 MPa) conditions. Germination was less than 10% when water stress was high (i.e. -1.0 MPa) with no germination after this point.

Unlike *A. setacea, A. caespitosa* (Dc1) was able to withstand lower water potentials when seeds remained in the florets, particularly under ionic stress (NaCl) at -0.5 MPa (P<0.001) and when water availability was extremely limited i.e. -0.75 MPa (P<0.001) and -1.0 MPa (P<0.001) (Fig 11b).

There were significant differences in germination of *B. macra* seeds under osmotic (PEG) and ionic (NaCl) stress (P=0.002). Under osmotic stress seeds germinated more successfully when cleaned (P<0.001) but under ionic stress germinated better, even when water was only moderately limiting (-0.25 MPa) (P<0.001) when seeds remained within the florets (Fig 11c).

Final germination percentages were significantly different between intact florets and cleaned seeds (P<0.001) in *C. truncata*. Germination was more successful under osmotic conditions (P=0.047) when seeds remained within the florets but overall germination was higher in both osmotic and ionic conditions when seeds were cleaned. Compared to the clean control seeds, however, germination was low (i.e. <40%) when water potentials were less than -0.25 MPa (Fig 11d).

Germination of intact *E. acicularis* seeds was less than 40% in the control seeds and less than 20% under low water stress conditions, which is significantly less than final germination percentages of cleaned seeds (P<0.001). The control cleaned seeds germinated up to 87%, this was reduced to 65% under moderate water stress (-0.25 MPa, PEG) and 47% in ionic conditions (P<0.001). Germination at low osmotic water potentials of -0.5 MPa (P=0.029) and -1.0 MPa (P=0.004) were significantly higher than seeds germinated in ionic conditions (Fig 11e).

Seed germination of *M. stipoides* was >85% up to -0.5 MPa in both osmotic and ionic conditions. Differences in germination percentages occurred when water stress was moderate-high (i.e. -0.75 MPa), in these conditions germination was >80% except when clean seeds were exposed to NaCl achieving 63% (<0.001). Osmotic conditions were less detrimental to germination in both intact and clean seeds and >80% germination could still be achieved at very low water availability (-1.0 MPa). At -1.0 MPa ionic conditions were unfavourable with only 47% germination achieved in cleaned seeds, keeping the florets intact at this low water potential improved germination up to 70% compared with clean seeds. At water potentials below -1.0 MPa germination was inhibited (Fig 11f).

4.5.2 Germination under saline conditions

A. setacea germination was significantly reduced at -0.5 and 1.0 MPa compared to the control treatments (P<0.001). All germination treatments reached over 80% germination under non-saline conditions. In moderately saline conditions (-0.5 MPa) germination was reduced to <35% in the control and kinetin treated seeds, while gibberellic acid and salicylic acid appeared to improve the seeds ability to withstand saline conditions. In highly saline (-1.0 MPa) conditions germination was reduced to <10% in seeds treated with water or gibberellic acid. Kinetin and salicylic acid treatments improved germination to 23% and 25% respectively (Fig 12a).

Germination of *A. caespitosa* (Dc1) was >98% across all treatments under non-saline conditions. This reduced significantly in saline conditions (P<0.001). However, under moderately saline conditions germination was still high at >85% across all treatments and 97% in salicylic acid treated seeds, which was significantly higher that the control seeds (P=0.015) and gibberellic acid treated seeds (P=0.017). In highly saline environments germination was around 50% across all treatments (Fig 12b).

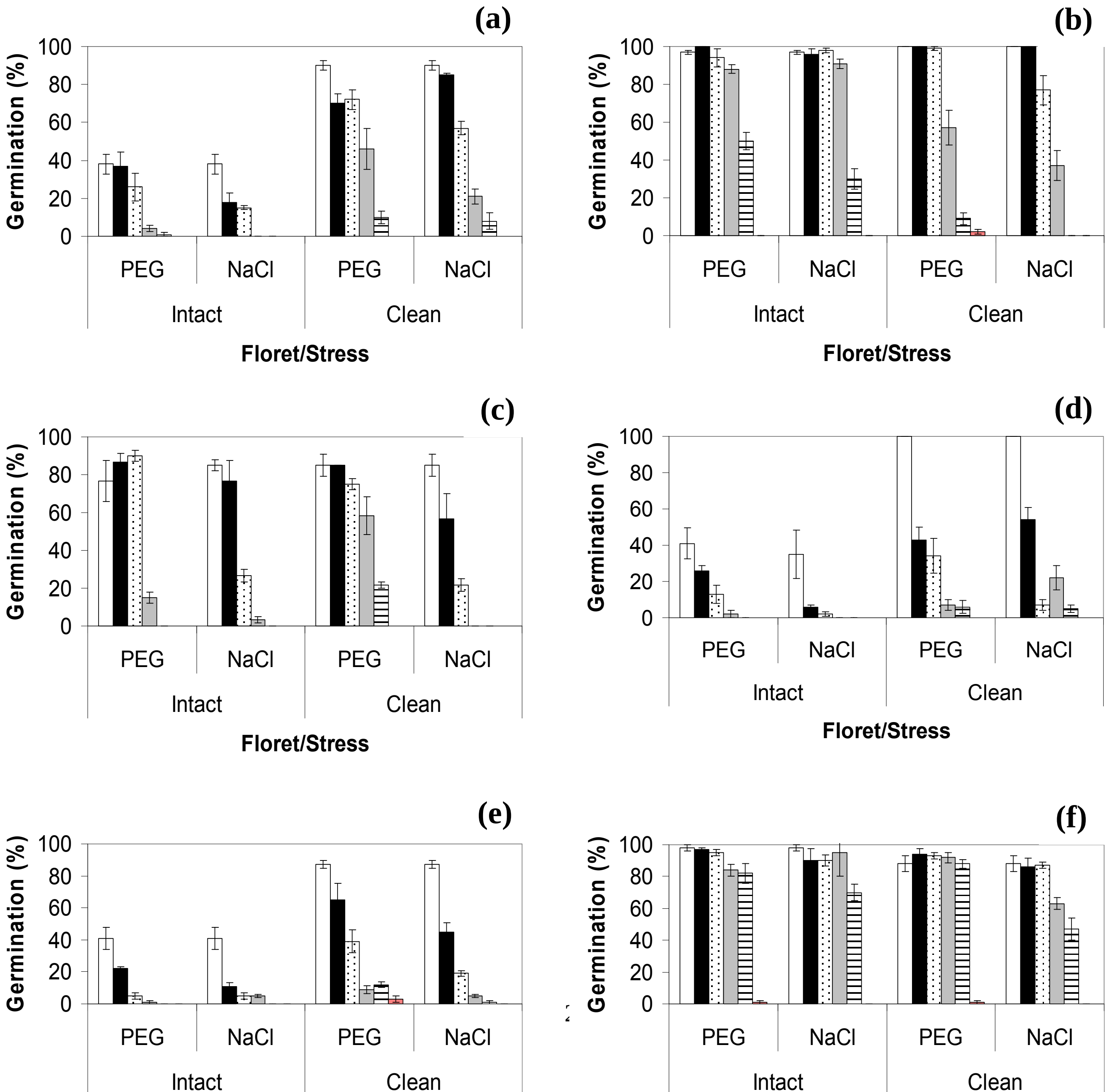

(a)
(b)
(c)
(d)
(e)
(f)
Germination (%)
0
20
40
60
80
100
PEG
NaCl
PEG
NaCl
Intact
Clean
Floret/Stress

Figure 11: Influence of water stress levels on percent seed germination in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Bothriochloa macra,* (d) *Chloris truncata,* (e) *Enteropogon acicularis,* (f) *Microlaena stipoides* (Lig 183). Water stress was imposed by PEG = Polyethylene Glycol, NaCl = Sodium Chloride; Intact = florets intact, Clean = caryopses removed from florets. Germination % indicated by bar columns; white = 0 water potential (ψ), black = -0.25 ψ, white/black dots = -0.5 ψ, grey = -0.75 ψ, white/black stripes = -1.0 ψ, orange = -1.5 ψ.

Chloris truncata appears to be the most saline sensitive species tested. In non-saline conditions germination was >90% across all treatments. In moderately saline conditions low germination of seeds treated with water only was enhanced with gibberellic acid (P=0.017), kinetin (P<0.001) and salicylic acid (P<0.001). Salicylic acid improved germination from 17% to 80%, while gibberellic acid and kinetin improved germination by 34 % and 50% respectively. Germination in highly saline conditions was <5% (Fig 12c).

In non-saline conditions gibberellic acid significantly improved germination of *M. stipoides* compared to the control (<0.001), kinetin (P<0.001) and salicylic acid (P<0.001) treated seeds. In moderately saline conditions gibberellic acid, kinetin and salicylic acid improved germination by up to 90% compared to the control (62%). Water treated seeds in highly saline conditions was low at 16%, which was improved significantly by gibberellic acid (70%; P<0.001), kinetin (55%; P=0.002) and salicylic acid (72%; P<0.001) (Fig 12d).

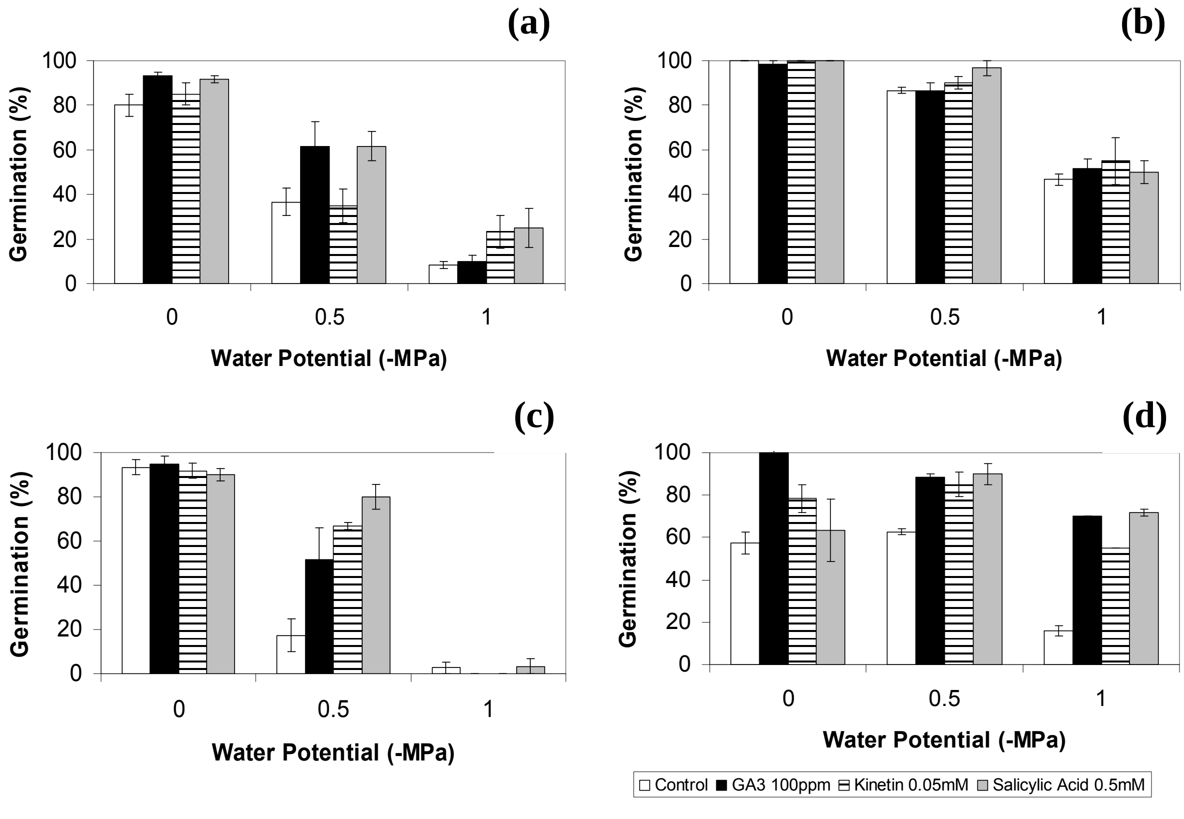


Figure 12: Effect of stress tolerance compounds on seed germination under saline conditions in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata,* (d) *Microlaena stipoides (Lig 183).* Water potential ψ; 0 = control, -0.5 ψ NaCl, -1.0 ψ NaCl. Germination % indicated by bar columns; white = water only (control), black = gibberellic acid 100ppm, white/black stripes = Kinetin 0.05 mM, grey = Salicylic acid 0.5mM.

4.5.3 Germination under water stress using plant signalling compounds

Germination of *A. setacea*, *A. caespitosa* (Dc1) and *E. acicularis* seeds treated with priming combinations was >80% across all treatments in both water potentials with no major benefit from combining enhancement treatments (Fig 14a, b, d).

C. truncata germination at 0 MPa was 68% in control seeds, this was improved to 82% with gibberellic acid and >90% with all other treatments. Priming benefits were observed in -0.5 MPa conditions by combining gibberellic acid and kinetin (95%), salicylic acid (53%), gibberellic acid and salicylic acid (52%) and gibberellic acid, kinetin and salicylic acid (50%). Control seeds germinated to 10% and all other treatments only achieved up to 20% (Fig 14c).

M. stipoides germination was 100% in control seeds in both 0 and -0.5 MPa and there was no difference in how seeds responded to priming treatments between 0 MPa and -0.5 MPa (P=0.112). Germination was significantly reduced in the priming combination treatments (except kinetin + salicylic acid) to <80% at 0 MPa (P<0.001), while single priming treatments (i.e. gibberellic acid, kinetin and salicylic acid) germinated to around 90% (Fig 14e). Similarly, germination was significantly reduced in the priming combination treatments (except kinetin + salicylic acid) to <77% at 0 MPa (P<0.001), while single priming treatments (i.e. gibberellic acid, kinetin and salicylic acid) germinated to around 85% (Fig 14e).

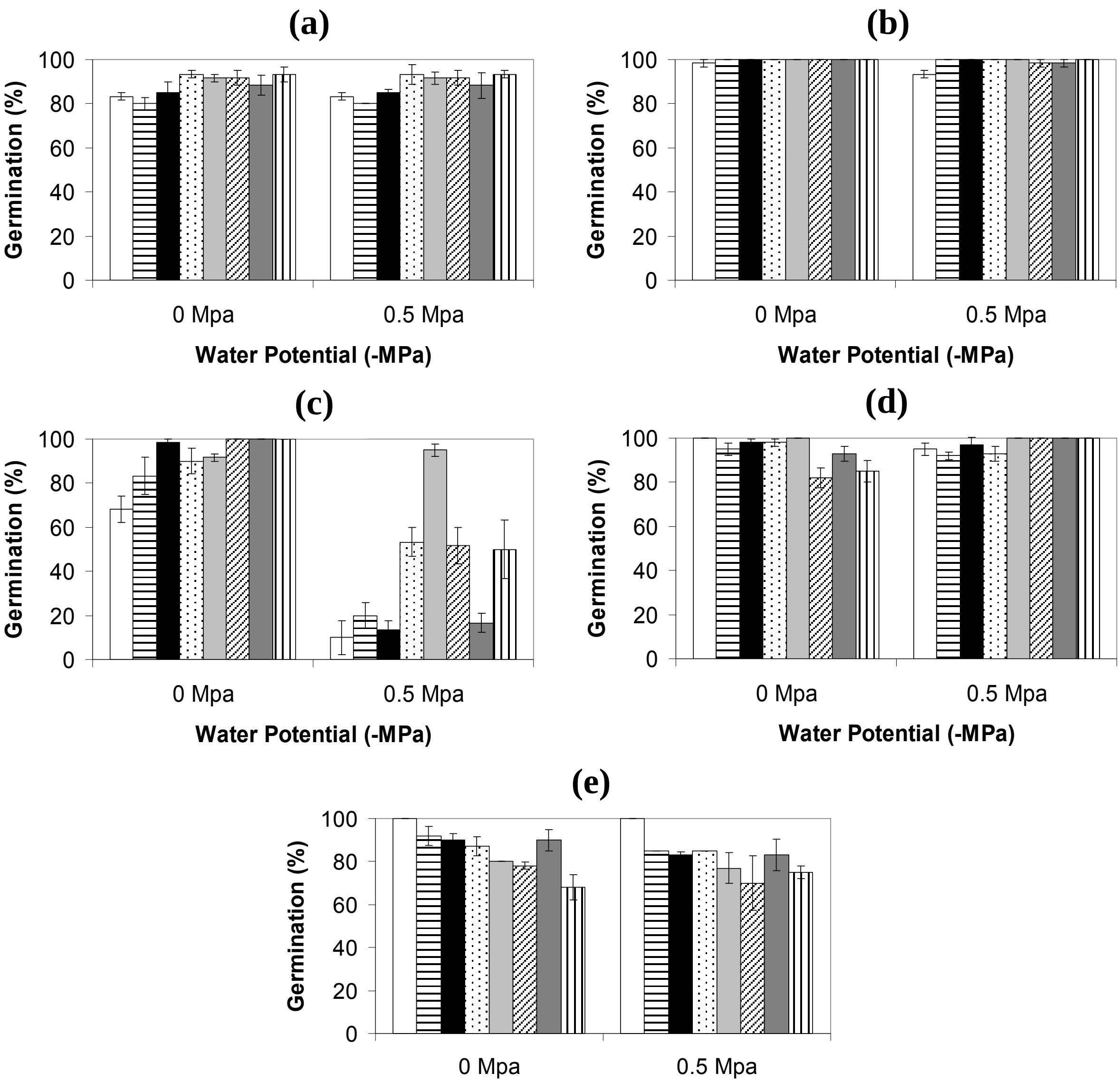


Figure 13: Overcoming water stress using seed priming treatment combinations in (a) *Austrodanthonia setacea*, (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata,* (d) *Enteropogon acicularis,* (e) *Microlaena stipoides* (Lig 183). Water stress was imposed using PEG to a water potential of either 0 ψ = control, or -0.5 ψ PEG. Treatments include; white = water only (control), white/horizontal black stripes = gibberellic acid 100ppm (GA), black = kinetin 0.05mM (K), white/black dots = salicylic acid 0.5mM (SA), grey = GA + K, white diagonal black stripes = GA + SA, dark grey = K + SA, white/vertical black stripes = GA + K + SA.

4.6 Seed Coating Technology

4.6.1 Laboratory Trial

Under controlled laboratory conditions film and polymer pellet coating did not provide any extra benefit to seed germination in either intact or cleaned seeds in any of the species trialled (Fig 14). Seed coating decreased germination in *A. caespitosa* Dc1, however no difference was evident in the other *A . caespitosa* line. Similar decreases were observed in *C. truncata* intact seeds (Fig 14c) and *M. stipoides* clean seeds (pellet coat) (Fig 14 e). Under no circumstance was GA detrimental to germination and in some instances (*A. caespitosa* cleaned pellet and *M. stipoides* intact filmcoat) provided a significant increase in germination (Fig 14).

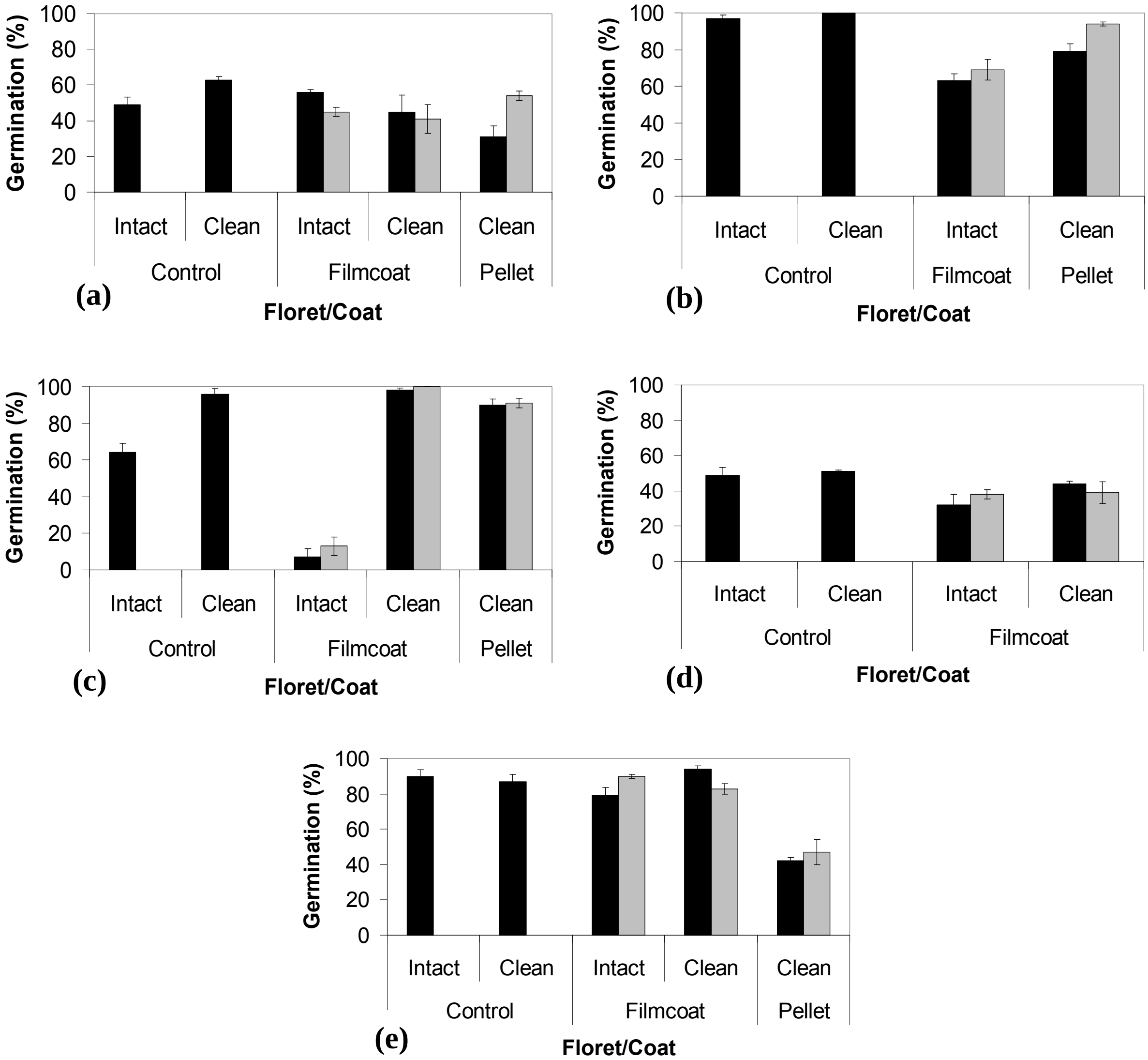


Figure 14: The influence of seed coating on germination under laboratory conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata,* (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.

4.6.2 Glasshouse Trial

Germination of surface sown seeds of *A. caespitosa* (WA wild stand collected – Bod 12/05) and *A. caespitosa* (Dc1) was higher than seeds sown at 1 cm depth. Film and polymer pellet coating did not improve germination compared to the control seeds (Fig 15 a, b). The highest germination achieved in *A. caespitosa* (Bod 12/05) when surface sown was 74% for intact control florets and 69% for cleaned control seeds. Germination at 1 cm sowing depth was <5% across all treatments (Fig 15a). *A. caespitosa* (Dc1) germination reached 95% in intact control seeds and 93% in cleaned control seeds that were surface sown. Germination of seed coated either by film and pellet coating had <90% germination when surface. Intact control seeds sown at 1 cm had very poor germination (i.e. <40%), and cleaned control seeds were much worse (8.5%) (Fig 15b). This was not improved by seed coating.

Germination of cleaned seed across all surface sown treatments in *C. truncata* was greater (i.e. >60%) compared to intact seeds in both surface and 0.5 cm sown seeds. Germination of seeds remaining in the floret was very poor (<10%) across all treatments (Fig 15c). Seed coating did not appear to have a significant negative impact on *C. truncata* germination.

D. sericeum germination was enhanced from 30% in the intact control surface sown seeds to 34% when seeds were coated with a film containing gibberellic acid (P <0.05). Cleaned seeds also benefited from gibberellic acid film coating and germination improved from 14% in the control to 18% with coating. Seeds sown at 1cm had very poor germination (<7%) across all treatments (Fig 15d).

Surface sowing seeds of *M. stipoides* was more successful than sowing seeds at 1cm depth under glasshouse conditions. Germination of intact seeds was >70% across all treatments, except clean, film coated seeds sown at 1cm where only 3.5% was achieved. The highest germination of intact florets was 87% in the control sown at 1cm. Cleaned surface sown control seeds were improved from 85% to 91% when film coated (Fig 15e).

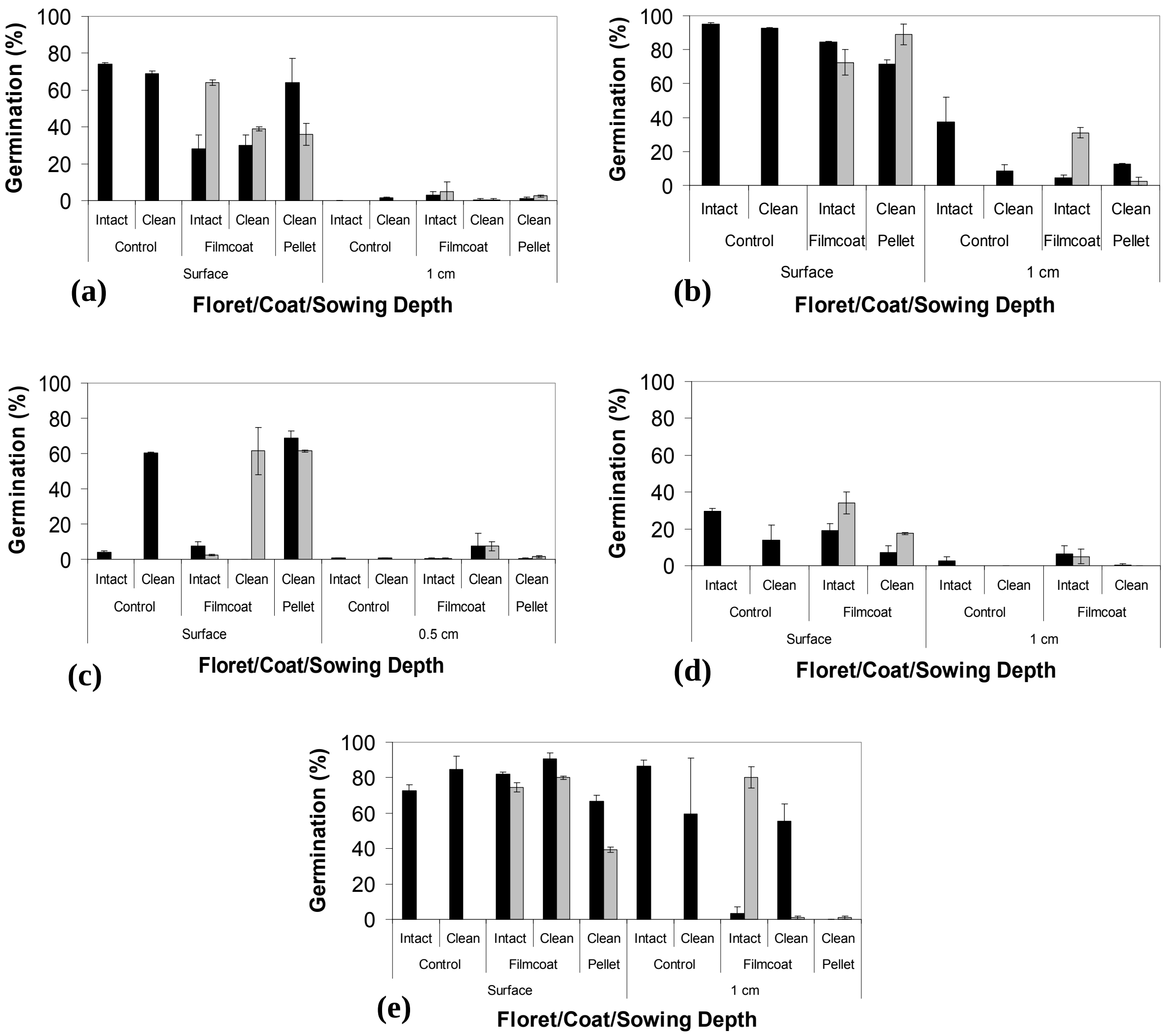
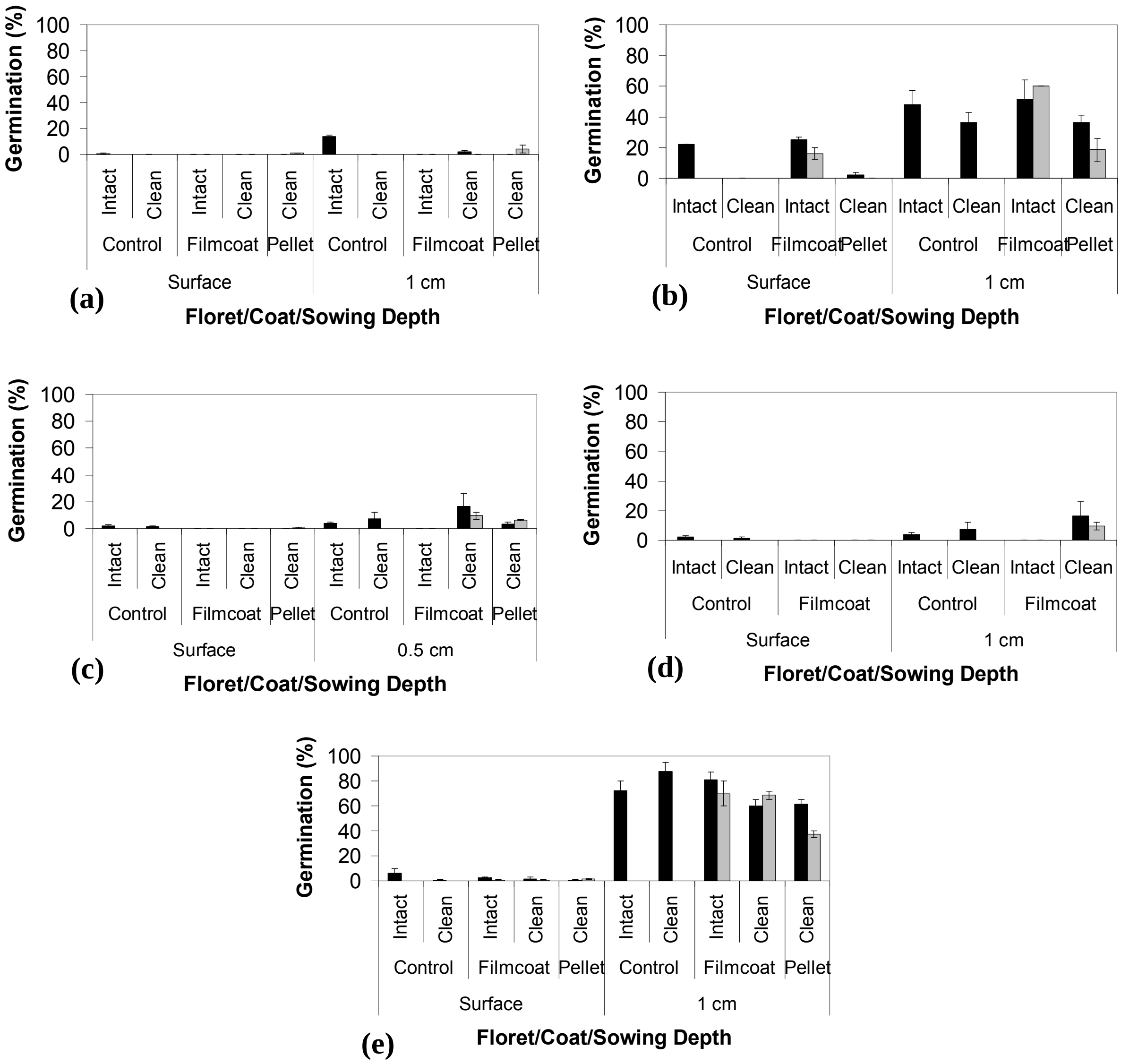


Figure 15: The influence of seed coating on germination under glasshouse conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata,* (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.

4.6.3 Field Trial

Seed coating technology did not improve germination of *A. caespitosa* (Bod 12/05), *C. truncata* or *D. sericeum* in the field trial (Fig 16 a, c, d). Germination across all treatments was either extremely poor or absent. *A. caespitosa* (Dc1) germinated better when sown at 1 cm compared to surface sowing. Germination was improved in intact control seeds from 22% to 48% by burying seed. Clean seeds that were surface sown had no germination; this was improved by 37% when seeds were buried. The best treatment was film coating with either water (52%) or gibberellic acid (60%) sown at 1 cm (Fig 16 b).

Surface sown seeds of *M. stipoides* had practically zero germination (the highest being intact control seeds with 6%) across all treatments. This was dramatically increased by sowing seeds at 1 cm depth. Intact control seed germination improved by 67% and clean control seeds by 87%. The best treatments were film coated intact seeds (81%) and clean control



seeds (88%) (Fig 16 e).

Figure 16: The influence of seed coating on germination under field conditions in Intact (florets intact) or Clean (caryopses removed from florets) of (a) *Austrodanthonia caespitosa* (Bod 12/05), (b) *Austrodanthonia caespitosa* (Dc1), (c) *Chloris truncata,* (d) *Dichanthium sericeum*, (e) *Microlaena stipoides* (Lig 183). Seedcoats included either a filmcoat = thin film ± germination enhancement treatment, Pellet = thick, shape changing polymer coat ± germination enhancement treatment. Treatments included; black = water only, grey = gibberellic acid 100ppm.

5. Discussion

5.1 Germination Enhancement

Improvements in germination by seed treatments in this study were shown to be species, accession and indeed collection location specific. Despite this, there appears to be several seed treatments that provide a general benefit to several species. Under controlled conditions the removal the caryopses from florets increases germination. This is in agreement with previous research that indicates the protective structures of native grass seeds can contribute to seed dormancy in some species (Tothill 1977; Lodge & Whalley 1981; Silcock *et al*. 1990; Adkins *et al*. 2002; Lodge 2004). Floret removal in the present study involved careful seed dissection by hand or by rubbing out with soft rubber mats. If naked caryopses are to be utilised in broad acre restoration, techniques need to be developed to scale up seed cleaning. This will be of particular importance for seeds like *M. stipoides* that require extreme caution in floret removal but which will return significantly increased germination (Fig 5c).

Priming technologies satisfactorily overcame seed dormancy under controlled environment conditions. For example seeds primed with gibberellic acid promoted the germination of 22 accessions (10 species) out of the 53 accessions tested (Table 2). Gibberellic acid is a growth hormone that promotes the elongation of cells and can stimulate germination or break dormancy in seeds that normally require after-ripening, or are physiologically dormant. Smoke water also promoted germination in 14 accessions (7 species) of native grasses tested. Smoke has been shown to have a similar mode of action to GA in stimulating germination of native species. Previous research indicates that some native grass species respond well to smoke treatments (Read & Bellairs 1999; Clarke & French 2004) and can overcome physiological dormancy (low growth potential of the embryo), a characteristic of the Poaceae family (Baskin & Baskin 1998).

Two of the C4 species (*Chloris truncata* and *Themeda triandra*) responded to a heat shock treatment, which is known to overcome after ripening requirements of seeds. Research has shown that continuous summer temperatures (32°/11°C day/night temperature) over three weeks is optimal for germination of C_4 summer growing species (Hagon & Groves 1977). Further research is required to determine whether exposure to high temperatures (i.e. 80 - 100°C) for short periods can also overcome after-ripening in other C_4 native grass species.

The ability to treat large amounts of seed with this priming technology is an important next step in optimising seed quality for use in broad acre restoration. The principles behind the priming process are simple. However, optimising the process for industry (cost, time, environmental conditions) still needs to be done if the process is to be practical.

5.2 Field Trials

5.2.1 C3 Species

In this study germination was improved under laboratory, glasshouse and field experiments compared to the industry standard of sowing seeds intact on the soil surface. The field study focussed *A. caespitosa*, and *M. stipoides* due to insufficient seeds of other species. Under laboratory conditions treatments improved final germination percentage above 80% in both of the species and in all accessions tested. Germination under glasshouse conditions was slightly less than under laboratory conditions however; germination was increased from 53% to 75% in *A. caespitosa*, 58% to 64% in *M. stipoides* (Griffin) and from 61% to 73% in *M. stipoides* (Ligule 183) with either seed cleaning or GA being the most consistent treatments. Improvements in final germination percentage under field conditions of up to 53% and 55%

were achieved for *A. caespitosa* and *M. stipoides* respectively. The best bet treatment for *A. caespitosa* was determined to be GA priming intact seeds buried at 1cm and for *M. stipoides* the best bet treatment was to have cleaned seed buried at 1cm (Fig 5).

From the results it is clear that the field surface sown seeds do not germinate as successfully as seeds that are buried at 1cm depth. Intact florets germinate better in the field than naked caryopses when surface sown, possibly due to the floret appendages retaining moisture resulting in more favourable seed water relations for germination. Likewise, soil water relations in glasshouse studies were likely to be more favourable for germination compared to field studies where soil drying from wind and sun will be more prevalent. This may partly explain the higher germination of surface sown naked caryopses in the glasshouse. Future research will need to focus on soil conditions that can optimise seed water relations or on improving seed germination performance under water stress conditions.

5.2.2 C4 Species

Germination was improved under laboratory, glasshouse and field experiments (Table 2). Under laboratory conditions, treatments improved final germination percentage above 60 % in *C. truncata* (cleaned seed plus GA priming) and above 90% in *D. sericeum* (cleaned seed plus GA priming) (Table 2). Germination under glasshouse conditions was slightly less than under laboratory conditions, however germination was increased from 41% to 86% in *C. truncata* (GA or KAR_1 primed cleaned seed sown on the surface). Under field conditions, final germination percentages of up to 29% were achieved for *C. truncata.* The best bet treatment was priming heat treated seeds with KAR_1 prior to burial compared to the control of 12%. GA was also another treatment that showed potential (up to 25% germination). For *D. sericeum* field emergence up to 67% was observed for GA treated intact seeds sown at 1cm. This resulted in a germination rate almost 2 fold greater than any other treatment.

From the results it is clear that under glasshouse and field conditions *C. truncata* germination and emergence can benefit from seed cleaning, surface sowing and priming with gibberellic acid or karrikinolide. Unlike other trialled species, *Chloris truncata* floret appendages do not appear to protect the seed from desiccation under field conditions, possibly due the a comparatively small seed size requiring less water to enable seed imbibition and germination allowing for immediate cell elongation and growth. Also, with the benefit of germination enhancers such as gibberellic acid and karrikinolide the speed of germination is further increased.

Interestingly, *D. sericeum* performed more similar in the glasshouse and the field compared to other species. Intact florets treated with gibberellic acid were by far the most successful combination for improved germination and emergence. In the glasshouse sowing depth did not appear to influence germination success but under field conditions burying seeds was preferable. Under field conditions soil moisture relations may be more favourable within the soil with less risk of drying from wind and sun, which may explain why glasshouse seeds germinated successfully whether surface or 1cm sown as the drying effects are minimised due to the protected conditions. Also, removing seeds from the florets has the potential to damage the caryopses minimising the ability for seeds to germinate successfully.

The interaction between soil traits (moisture retention and drying susceptibility) and seed treatments is likely to be an important factor in determining the suitability of these treatments for broad scale usage. Like the species used in the C3 trial, future research will need to focus on soil conditions under water stress but also optimising seed-soil water relations (i.e. interactions of soil type, sowing depth and climate) and how this may influence the success and speed of germination and emergence.

5.3 After-ripening and storage

All four species possessed at least partial dormancy before being placed in after-ripening conditions. Under these conditions *T. triandra* and *D. sericeum* did not show improvements in germination over the 3 month period. *A. setacea* was reduced after 1 month and *C. truncata* germination was reduced after 2 months in these conditions (45^{o}C, 50% RH). For some species (*A. setacea, C. truncata, D. sericeum* and *T. triandra*) the after-ripening conditions utilised were limited to one temperature and RH% that were thought to induce rapid aging of the seeds. It is likely that other after-ripening conditions may be more suitable (i.e. less detrimental over the short term), and provided seed is not a limiting factor, curves should be developed for a range of temperatures and RH's to identify optimal after ripening conditions. The fact that there are species differences under these extreme storage conditions highlights the importance of treating each species separately.

For the two species *M. stipoides* and *A. caespitosa* other RH's and storage temperatures were examined. Seeds of *A. caespitosa* (Dc1) stored at temperatures of 5°C and -18°C and 50% RH appeared to benefit from 6 months storage, while germinability was reduced in seeds stored at 23°C. The most significant finding was that cleaned fresh *A. caespitosa* seeds maintained a significantly higher germination potential than intact seeds up to 6 months after storage at -18 ^{o}C, 5 ^{o}C and 23 ^{o}C at both 20% and 50% RH. Cleaned seed viability, however, declined at all storage temperatures after 6 months at 20% RH. *M. stipoides* viability remained high after 12 months at low temperatures with some decline in intact seeds and a significant loss in viability in cleaned seeds stored at 23°C, 50% RH.

After-ripening has shown to be important in species that were thought to possess deep dormancy. For example, Wells and Dixon (2003) found that *Triodia* species, once thought to be deeply dormant, were able to germinate when seeds were stored for 12 months in ambient conditions. It appears that seed dormancy and after-ripening requirements are species specific, requiring particular conditions before dormancy is broken. Groves *et al* (1982) found that populations of *Themeda australis* collected from across Australia had different levels of dormancy between populations, attributed to maternal environment effects.

Future research should be directed towards determining whether genetic, or maternal environmental conditions of native perennial grasses are fundamental to successful germination and establishment. Also, accelerated after-ripening experiments using various temperatures and relative humidity could be implemented to determine variations between species and dormancy length. Previous research also suggests that alternating storage temperatures may facilitate germination in some Poaceae species, for example, Hagon (1976) found that *Themeda australis* and *Austrostipa* sp. germination could be increased substantially after seed storage in alternating warm or cold temperatures. Reducing moisture content is also an important factor associated with the optimal storage of seeds and research suggests that it is the most important determinant of longevity in storage (Wilson 1995, Merritt 2003).

5.4 Stress Tolerance

This study highlighted large differences between species in their ability to germinate under drought/saline conditions. *Microlaena stipoides* was shown to be extremely resilient to osmotic stress (induced by PEG) and salt stress (NaCl), whilst others like *E. acicularis* was determined to be water stress sensitive. Differences within genera were also observed with *A. setacea* being significantly more susceptible to salt than *A. caespitosa*. With the need to find salt and drought tolerant species for restoration of degraded agricultural lands some native grasses may offer a suitable alternative to other more studied plant species. A drought/salt tolerance screening process (particularly focussing on *M. stipoides*) should be considered to identify tolerant ecotypes, both at the germination phase and at the early seedling and adult phase. Given *M. stipoides* appears to have a robust tolerance to drought/salt at the early germination phase, determining the mechanisms of tolerance may be beneficial to identify key tolerance traits.

The tolerance of native grasses was also shown to be improved through priming seeds with stress tolerance agents including kinetin and salicylic acid. These compounds improved germination in 3out of the 4 native grass accessions (2 out of 3 species) tested. It appears that these chemicals provide a means to improve germination under drought conditions at minimal risk. As the mode of action of these chemicals in conferring stress tolerance is not known at present, more work is required to test the robustness of these treatments under field conditions before a final recommendation can be made.

5.5 Seed coating

In this study the ability of seed coats to deliver germination signals under controlled environment and field conditions was examined. Overall seed coatings were shown to produce germination results similar to uncoated seeds, indicating that germination stimulants supplied by artificial coats provided no added benefit to field establishment. That said, the role of seed coating in providing benefits to field establishment of native grasses should not be dismissed. The ability of seed coats to aid in seed handling may indeed result in more efficient plant establishment at much lower seeding rates/costs. Further research under a range of field conditions (soil types, environments) interacting with seed to site delivery mechanisms will demonstrate the role of seed coats in broad acre agriculture. If seed coats can not effectively transfer the necessary signals to the seed to promote germination, but are required for adequate seed handling a combination of seed priming and coating may be necessary, provided the system is simple and cost effective.

6. Implications

This research has direct implications for the native grass industry, particularly for suppliers and collectors of native seed. Relatively simple and cheap seed treatments produced significant improvements in native grass germination and were useful across many species/accessions tested. The approach used in this report can be readily adapted to industry to improve the quality of the end product. This quality increase will hopefully facilitate broad scale adoption of native grasses in Australia.

Best bet treatments for several key native grass species have been identified including:

- *A. caespitosa* - GA priming intact seeds buried at 1cm
- *M. stipoides* - cleaned seed buried at 1cm
- *C. truncata* - priming seeds with KAR_1 or GA prior to burial
- *D. sericeum* - GA treated intact seeds sown at 1cm.

If these findings are to be adopted for broad scale industry use, this technology needs to be scaled up ensuring end users are able to cost effectively treat their seed.

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8. Acknowledgments

This work was conducted by the Kings Park Botanic Gardens & Parks Authority as part of the project "*Grass roots – native perennial grasses for sustainable pasture systems*" funded by the Rural Industries Research and Development Corporation and Native Seeds Pty Ltd, with links to the project "*Reliable establishment of non-traditional perennial pasture species*", funded by the Future Farm Industries CRC (formerly the CRC for Plant-Based Management of Dryland Salinity), Meat & Livestock Australia, Australian Wool Innovation and Land and Water Australia. In-kind support was provided by the Department of Primary Industries Victoria, Queensland Department of Primary Industries, the University of Western Australia and the Department of Agriculture and Food Western Australia. Seed Solutions Pty Ltd provided access to seed coating technologies and incorporated chemicals into seed coats for this research.

9. Abbreviations

A1108	*Microlaena stipoides* (Griffin) Harvest date: 03/08
A2108	*Microlaena stipoides* (Griffin) Harvest date: 03/08
B1108	*Microlaena stipoides* (Griffin) Harvest date: 03/08
B1208	*Microlaena stipoides* (Griffin) Harvest date: 03/08
Bod	Boddington (Perth Hills) Harvest date: 12/05
C3	winter growing grass
C4	summer growing grass
D11a, b	*Dichanthium sericeum* - Department of Primary Industries, QLD, ecotype 11
D16a, b	*Dichanthium sericeum* - Department of Primary Industries, QLD, ecotype 16
Dc1	*Austrodanthonia caespitosa* - Cathy Waters line, Trangie NSW
Dr1	*Austrodanthonia racemosa* – Monaro NSW
DRfl	*Austrodanthonia fulva* – Monaro NSW
GA	gibberellic acid (100 parts per million)
H2O	water
K	kinetin (0.05 mM)
KAR_1	karrikinolide (100 parts per billion)
K20a, b	*Themeda triandra* - Department of Primary Industries, QLD, ecotype 20
K24a, b	*Themeda triandra* - Department of Primary Industries, QLD, ecotype 24
LiCl	lithium chloride
Lig 183	*Microlaena stipoides* – Ko-warra Native Grasses, Euchla
MPa	measurement of water potential in megapascals
NaCl	sodium chloride
NS281	*Chloris ventricosa* (Barwon) 02/2008 – Myrtleford (Native Seeds Pty Ltd)
NS285	*Chloris truncata* (Common) 02/2008 – Myrtleford (Native Seeds Pty Ltd)
NS289	*Microlaena stipoides* (Ovens) 03/2008 – Wangaratta (Native Seeds Pty Ltd)
NS290	*Microlaena stipoides* (Bremmer) 03/2008 – Wangaratta (Native Seeds Pty Ltd)
NS292	*Microlaena stipoides* (Griffin) 03/2008 – Wangaratta (Native Seeds Pty Ltd)
NS418	*Bothriochloa macra (*Common) 03/2008 – Tamworth (Native Seeds Pty Ltd)
NS449	*Microlaena stipoides* (Griffin) 12/2006 – Wangaratta (Native Seeds Pty Ltd)
PEG	polyethylene glycol
RH	relative humidity
SA	salicylic acid (0.5 mM)
SC204	*Austrodanthonia caespitosa* – Northam, WA 12/2006 (Sam Clarke)
SC206	*Austrodanthonia caespitosa* – Kellerberrin, WA 12/2006 (Sam Clarke)
SC210	*Austrodanthonia sp. Goomalling* – Quairading, WA 12/2006 (Sam Clarke)
SC217	*Chloris truncata* – Grass Valley, WA 12/2006 (Sam Clarke)
SC219	*Austrodanthonia pilosa* – Armadale, WA 12/2006 (Sam Clarke)
SC222	*Austrodanthonia setacea* – Mundaring, WA 12/2006 (Sam Clarke)
SC226	*Chloris truncata* – Miling, WA 10/2007
SUIN	surface sown, intact florets
SUCL	surface sown, clean seeds
SW	smoke water (1% solution)
S8a	*Heteropogon contortus* - Department of Primary Industries, QLD, ecotype 8
1cm IN	1cm sowing depth, intact florets
1cm CL	1cm sowing depth, clean seeds
Ψ	water potential

Low Cost Rehabilitation of Native Perennial Grass Pastures by Managing Seedling Recruitment

Future Farm Industries CRC Technical Bulletin

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Native pasture of wallaby grass (*Austrodanthonia* spp.) during flowering at Trunkey Creek, central New South Wales, in November 2007. (Photo: R. Thapa)

Contents

1. Abstract

Many permanent pastures across southern Australia have sub-optimal levels of perennial grass species, below that required for sustainability. However, increasing perennial grass content by conventional re-sowing is often unprofitable. Research in central New South Wales, with the native grasses, wallaby grass (*Austrodanthonia* spp.) and red grass (*Bothriochloa macra*), has identified low-cost, ecologically-based management options to enable the successful recruitment of native perennial grass seedlings within existing pastures. Successful recruitment occurs in early autumn, provided the following conditions are met: (i) adequate quantities of germinable seed are available (following removal of stock from spring to promote flowering and seed set); (ii) a suitable rainfall event after seed maturation and shedding results in a 7-15 day period of moisture in the top 50 cm of soil; and (iii) an appropriate micro-site is present for seedling emergence (light scarification can help on soils that are not self-mulching). Insecticides can be used to control seed-harvesting ants and sub-lethal herbicide doses can be used at seed maturity to weaken plant competition prior to recruitment. Climate analyses indicated that soil moisture conditions in February-March should be favourable for seedling recruitment in most years. These results have been used to develop a management package for farmers to enhance the recruitment of desirable perennial grass seedlings within existing pastures. The techniques can be used to significantly increase the perennial grass content of pastures across south-eastern Australia at low-cost, with substantial impacts on medium to long-term profits and environmental benefits.

2. Background

The grazing industries in high rainfall, non-arable areas of south-eastern Australia have traditionally been reliant on native pastures for animal production. Kemp and Dowling (2000) suggest a paddock composition of at least 60% of desirable native grasses is needed to optimise pasture productivity and sustainability. Many pastures, however, have become degraded, with grass populations below sustainability levels. Kemp and Dowling (2000) found the current perennial content of many pasture systems is often ≤ 20% of pasture composition, well below the desired level. The loss of perennial grasses and their replacement by annual species has had severe implications for the productivity of livestock enterprises by increasing the risks of weed invasion, erosion, salinity and acidity and reducing biodiversity (Kemp *et al*., 2000; Michalk *et al*., 2003). Sustainability of these grasslands is of major concern to landholders and the community at large. A majority of farmers surveyed in the high rainfall temperate zones of Australia also believed it was worthwhile re-sowing old or degraded pastures, even though they may not always make an initial profit from them (Reeve *et al*., 2000). This is indicative of a productivity decline and the desire of farmers to restore paddocks to previous productivity levels.

Improving the perennial grass content of pastures has previously been attempted by sowing exotic species, but in many areas this is difficult, due to steep terrain, or is unprofitable. The average cost of $300/ha generally means 8-10 years are needed to recover costs (Vere *et al*., 1997; Bolger and Garden, 1998), which is particularly problematic on soils of low fertility and in low rainfall areas. Re-sowing also results in a short term loss of production while the newly sown pasture grows. With declining terms-of-trade for livestock production and increasing climate variability and its associated risks, the relative cost of re-sowing has become more marginal, except on more fertile soils and in higher rainfall environments where the chances of success are greater.

An alternative, lower cost method of pasture renovation is to manage perennial pastures in a way that allows successful recruitment of new plants of desirable species. This requires development of low-cost, ecologically-based management practices for existing pastures that contain desirable perennial grass species, by enabling the recruitment of seedlings from seed set by the existing plants. In this way perennial grass content can reach desired levels over time. Using such an approach is likely to be less costly and risky than re-sowing, particularly on less fertile soils and in low rainfall areas. Many degraded pastures still have a residual level of desirable perennial grasses that could be used as the basis for seed production and recruitment of new plants, provided methods to manage the process can be devised. Such procedures need to target: (i) the successful production of viable seeds; (ii) the successful delivery of those seeds to the soil surface for establishment to occur; and (iii) the successful recruitment and survival of young plants.

Recent national research programs have concentrated on increasing perennial grass content using tactical management (Kemp *et al*., 2000, 2003). Much of that work has developed effective management practices that maintain existing plants in a productive state, but has not provided insights into how existing plants are replaced through seedling recruitment (Kemp *et al*., 2000). The latter requires an understanding of the population dynamics of perennial species, which is poorly understood. Pastures based on perennial grasses can be sustained in the short-term through reducing grazing pressure and encouraging vegetative growth, but may fail to recruit new seedlings in the absence of suitable environmental and management conditions. The result is the degeneration of pastures over time.

Understanding how to encourage recruitment of new seedlings into existing swards is a substantial knowledge gap in grassland ecology. Limited research has shown that little, if any, desirable grass seedlings develop into mature plants in existing paddocks, reflecting a poor understanding of the mechanisms underlying the recruitment process. In a study of seed dormancy, germination, seedling emergence and survival of perennial pasture grasses in northern New South Wales, Lodge (2004) posed the challenge of identifying the causes that lead to limitations in successful recruitment. This clearly highlights the need for more research to devise appropriate and practical management options to encourage the emergence and survival of new seedlings into pastures. The work of Lenz and Facelli (2005) in South Australia on recruitment of both native and exotic perennial grass further identified the need for research to better understand the reasons behind the low survival of perennial grass seedlings.

This project investigated a three-phase approach to develop simple low-cost management procedures to enhance the content of desirable perennial grass species. Management strategies were designed to encourage recruitment by: (i) encouraging seed set and shedding by desirable species; (ii) preparation of more suitable micro-sites for seedling recruitment; and (iii) identifying the best post-emergence tactics to aid seedling recruitment in the short to medium-term. Research was conducted on the native grasses, red grass (*Bothriochloa macra*), a C4 species, and wallaby grass (*Austrodanthonia* spp.), a C3 species to develop general principles applicable to a range of native grass species.

3. Methodology

3.1 Design and treatments

This project involved a series of inter-related field experiments in central NSW, plus additional small scale studies to investigate specific issues. Climatic analyses were also conducted to assess the probability of the conditions resulting in successful recruitment occurring in practice.

The project focussed on field studies in the central tablelands and nearby slopes of central NSW, where native perennial grasses are widespread and important for grazing. The wallaby grass site was located at Trunkey Creek (33°49'S, 149°19'E) and the red grass site was located at Wellington (32°30'S, 148°58'E) (see Figure 1). C3 (wallaby grass) and C4 (red grass) species were included to investigate possible differences in responses between the two grass types. Field sites were selected with the aim of having a range of competing plant species including other perennial grasses, annual grasses (the most common competitors) and broadleaf species.

Experiments were conducted from 2006 to 2008. Treatments in the main field experiments were designed to investigate each of the key phases of seedling recruitment in sequence, with factorial combinations of seed delivery, viable seed levels and site preparation as treatment factors. All experiments were laid out in a randomised block design with 4 replicates. The wallaby grass experiments were repeated over two years. Only one year was possible at the red grass site due to its unavailability from the second half of 2008.

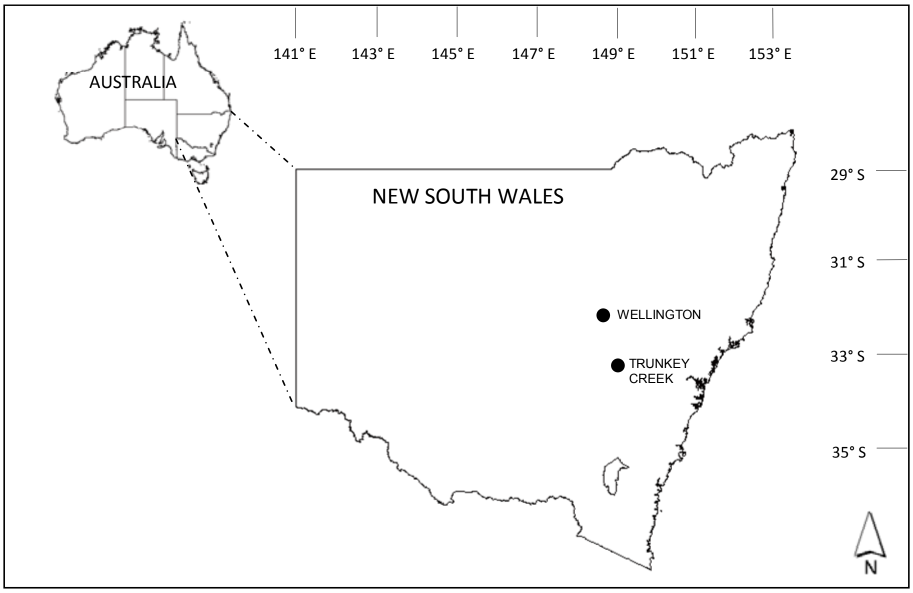


Figure 1. Location of experimental sites at Wellington and Trunkey Creek in central New South Wales (NSW), Australia. Grid references for NSW in latitude and longitude are shown.

The following treatments were conducted.

Treatment Factor A: Seed delivery mechanisms - pre-emergence phase

- **Cut and remove (CR):** Herbage mass was reduced to a level similar to grazing, by cutting to 20-50 mm above ground and removing plant matter. This aimed to simulate the physical movement from grazing that causes some seeds to drop to the ground, with others being consumed. *[At the red grass site CR was modified into a grazed (GR) treatment.]*
- **Grazed (GR):** The plots were grazed prior to sowing in May-June and again in November-December. It was assumed that grazing would cause some perennial grass seeds to drop to the ground, with others being consumed, hence altering the amount of seed available.
- **Pasture cropped (PC):** An area within a larger experiment that had been cropped in 2006 was used for this treatment. It was anticipated that soil disturbance from the PC treatment would increase the potential number of micro-sites for seedling recruitment through the availability of more bare ground and rougher soil surfaces. [*At the wallaby grass site, PC was replaced by SR within treatment factor C*]
- **Uncut control (UC):** The sward was left uncut. In the red grass experiment, this treatment was replaced by an ungrazed treatment (UG).

Treatment Factor B: Modifying viable seed levels

- **Insecticide application (IS):** Dead seeds treated with Gaucho ® insecticide (active ingredient: 600 g/L imidacloprid) were added at a rate of 50 kg/ha to limit predation from ants on existing viable seeds. Phalaris seeds were used, due to their attractiveness to ants (Campbell, 1966; Campbell and Gilmour, 1979). *[IS was dropped at the red grass site because ants were not a major problem (W B Badgery, pers. comm.).]*
- **Seed addition (SA):** Extra seeds (50 kg/ha) were added to test if recruitment was seed-limited.
- **No seed addition control (NS):** No seeds were added.

Treatment Factor C: Site preparation and reduced competition

- **Herbicide application (HA):** A sub-lethal dose (250 mL/ha) of the grass-selective herbicide, Fusilade ® (active constituent: Fluazifop-P), was applied to weaken competition from adult plants and to kill any germinating annual grasses.
- **Scarify and rake (SR):** Approximately 50% of the plot area was lightly scarified by hand raking to remove small competitors and existing adult plants. This was done to create more bare ground and rougher soil surfaces as potential micro-sites for germination and seedling recruitment. This treatment aimed to simulate the soil disturbance created by cattle and sheep hooves during grazing or through light tillage. This treatment also changed the location and density of litter biomass. *[At the red grass site SR was removed, as it was incorporated within the pasture cropping treatment in treatment factor A.]*
- **No preparation control (NP):** The plot was left unmodified.

Native pastures at the experimental sites on October 2007. Left: Wallaby grass at Trunkey Creek, right: red grass at Wellington. (Photos: R. Thapa)

3.2 Data collection and analyses

Field sites were characterised in terms of botanical composition and soils. Climate data were collected with data loggers at each site for the duration of experiments.

Field experiments focused on fine-scale effects on plant recruitment. Measurements were conducted inside 0.9 m x 0.9 m permanent quadrats, located in the centre of 2 m x 2 m plots. The 0.9 m x 0.9 m areas were subdivided into nine 0.3 m x 0.3 m contiguous quadrats (arranged in a 3 x 3 square); these were used for measurements of botanical composition. Each of these quadrats was further divided into 3 x 3 quadrats, each 0.1 m x 0.1 m, in which seedling numbers were recorded. Treatments were applied over the whole 2 m x 2 m plot areas.

Dry weight ranks of the three most abundant species and the total dry matter (DM) of all species were estimated using BOTANAL procedures (Tothill *et al*. 1992). Weights of standing DM (t/ha) and litter DM (t/ha) were estimated separately. Sampling for pasture biomass was done each year in late summer (February), autumn (May), winter (August) and spring (November). Plant cover, litter cover and bare ground percentages were visually estimated. Initial seedling counts were conducted within 2-3 weeks of seeds germinating. Counts were repeated 6, 24, and 52 weeks later. Within the plot, two soil cores (0.05 m diameter x 0.05 m deep) were randomly collected - one from each side of the 0.9 m x 0.9 m area used for measurements. These were taken back to the glass house for seed bank studies at the start of each experiment. The number of plants and seed heads were counted in ten randomly selected 0.3 m x 0.3 m quadrats at the time of seed maturity. Seed heads were collected from ten of these plants and the seeds counted to estimate the number of seeds per head and total seed production.

Statistical analyses were conducted using Systat and Genstat. Spatial techniques were used to analyse seedling density data, as there were significant gradients in recruitment rates across sites that were not related to treatments.

Measurement quadrats on April 2007 for biomass (left) and seedling counts (right). (Photos: R. Thapa)

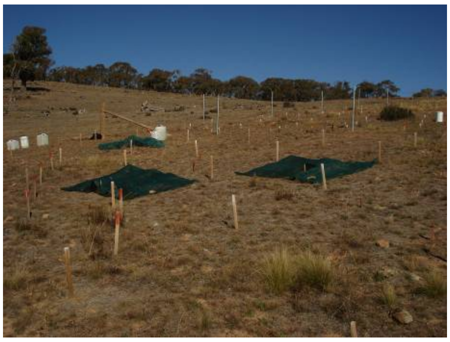
3.3 Irrigation experiments

Irrigation experiments were designed to investigate germination of the seed bank throughout the year and were conducted at the same field sites in Trunkey Creek and Wellington. The experiments started in January 2007 and continued until October 2007 at Wellington and until February 2008 at Trunkey Creek.

Plots measured 1 m x 1 m and treatments were replicated 3 times. A 1 m x 1 m galvanised metal plate (0.15 m high) was used to retain water within plots. Water was applied at 6-weekly intervals at a rate equivalent to 50 mm rainfall (50 L/m^2) over two days (25 mm each day). Plots were covered with shade cloth for 2-3 days following irrigation to reduce evaporation. Separate plots were used on each occasion.

The centre 0.9 m x 0.9 m of each plot was divided into 3 x 3 quadrats of 0.3 m x 0.3 m and each quadrat further divided into 6 x 6 sub-quadrats of 0.05 m x 0.05 m. Pasture yield and composition was measured within quadrats after each watering using BOTANAL procedures and emerged seedlings were recorded in two sub-quadrats (one for high and one for low emergence) two weeks after each watering. Two soil cores (0.05 m diameter x 0.05 m deep) were collected from each plot outside the central 0.9 m x 0.9 m measurement zone and grown for a 2-week period in the glass house for seed bank measurements.

Due to a lack of seedling emergence and accumulated evidence that the perennial grass soil seed bank was exhausted (Thapa 2010), the design was modified at Trunkey Creek to add the equivalent of 50 kg/ha of perennial grass to the watered plots from November 2007 to February 2008. There were 13 watering events, of which 5 events recorded recruitment. Of these, 3 events had both water and seed applied and coincided with rainfall events.

Irrigation plots (left) and logger for climate data (right), April 2007. (Photos: R. Thapa)

3.4 Soil moisture model

Moisture content (as percent volume) was estimated using the Sustainable Grazing Systems (SGS) pasture model (Johnson *et al*., 2003; using V 4.5.4, Johnson, 2008). The SGS model uses daily climate data (rainfall, temperature, relative humidity, wind speed, vapour pressure, evaporation, solar radiation), soil physical properties (based on the generic soil type), soil nutrient status (based on initial inorganic values for NO_3 and NH_4), pasture species and latitude to calculate soil moisture values. The analyses focused on soil moisture conditions in the top 50 mm created by rainfall events that started immediately before the time of identified recruitment events. Long-term data were estimated from the Datadrill® program that predicts climate data from the co-ordinates of surrounding weather stations (Jeffery *et al*., 2001).

4. Results

4.1 Wallaby grass experiments (Trunkey Creek)

Monthly rainfall and maximum and minimum temperatures at Trunkey Creek are shown in Figure 2 for the 2006-2008 experimental period, along with the previous 30-year averages.

4.2.1 First year (2007-08)

Wallaby grass seedlings germinated and established in early March 2007 after rainfall of 93 mm in the preceding month (Figure 2). Due to the dry season there was very limited seed set. The greatest seedling number (296/m^2) was observed where the sward was uncut, extra seed had been added and the soil surface layer had been disturbed through scarification and raking (Figure 3). Survival of emerged wallaby grass seedlings was very low. On average 24 seedlings/m^2 germinated at the site, but only 2 seedlings/m^2 remained six weeks after emergence. However, many of these seedlings survived through the year, with an average density of 1 seedling/m^2 at 52 weeks after emergence. The highest seedling survival (19/m^2) came from treatments where the sward was uncut, extra seed had been added and herbicide had been applied.

Wallaby grass had marginally more seedling germination in the cut and remove (CR) treatment than uncut (UC) treatment ($P<0.01$), possibly due to a non-significant interaction with seed addition treatments. Seed addition (SA) treatments significantly increased seedling numbers, compared to no seed addition (NS) or insecticide application (IS) treatments ($P<0.001$). Higher levels of seedlings were observed in the scarify and rake (SR) treatment compared to no preparation (NP) and herbicide application (HA). Within the SR plots, there were more seedlings observed in the IS and SA treatments than NS treatment ($P<0.01$). Overall, plots that combined the SA and SR treatments had the highest seedling establishment ($P<0.01$). Substantial seedling losses occurred after emergence in all treatments, but trends present soon after emergence were still evident six and 24 weeks later. At 52 weeks after emergence the SR and HA treatments had marginally higher plant densities ($P<0.01$).

Multiple regression analysis indicated seedling numbers were maximised where bare ground was limited (10-15 % of surface area), litter quantity was high (~0.5 t/ha DM) and green plant material was ~0.5 t/ha DM at emergence. At 24 weeks after emergence, higher plant cover (20-35%) resulted in maximum seedling survival. Significant relationships were not observed 52 weeks after emergence.

Wallaby grass recruitment responded in general to leaving swards intact, with positive effects from scarification and to a lesser extent to sub-lethal applications of herbicide. Maximising seed set was obviously critical as there was a low level of seed set and a generally low density of seedlings.

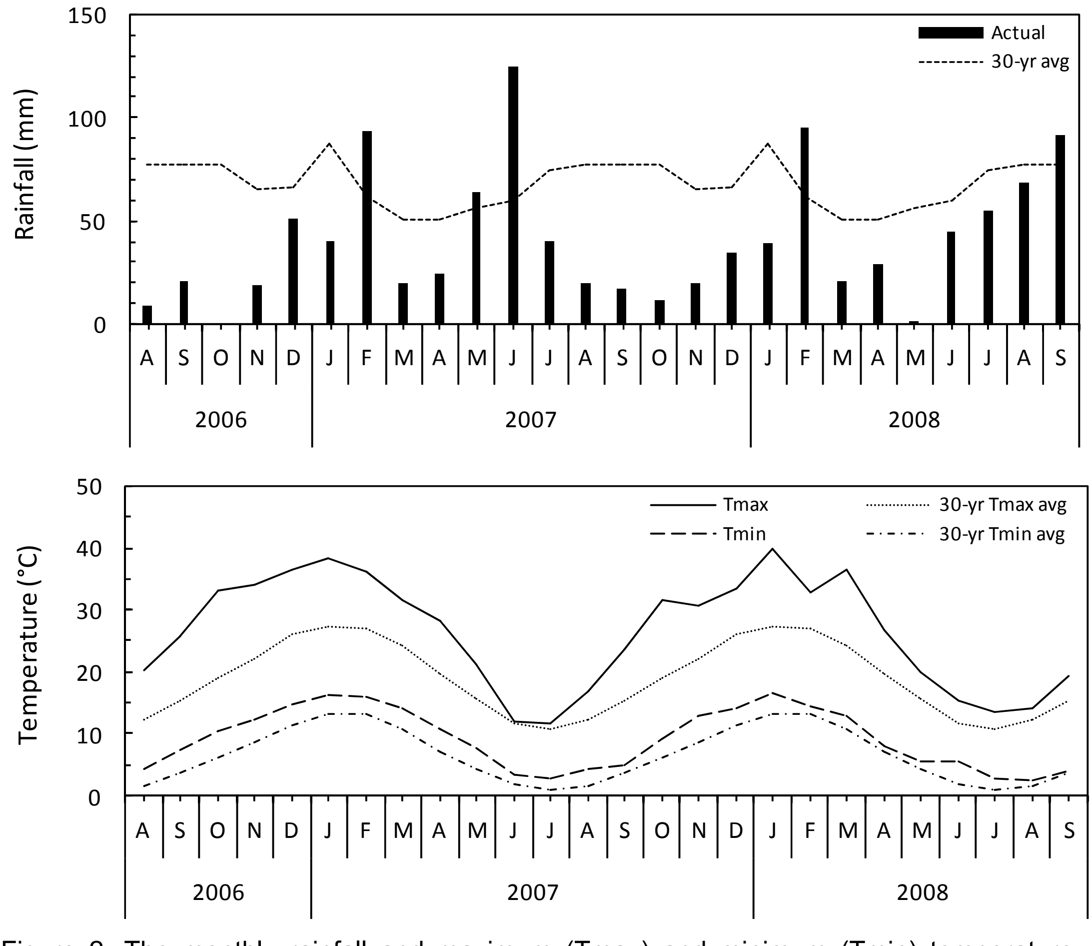


Figure 2. The monthly rainfall and maximum (Tmax) and minimum (Tmin) temperature at Trunkey Creek during the 2006-2008 experimental period plotted with the 30-year averages (1971-2000).

4.2.2 Second year (2008)

Wallaby grass seedlings were observed in early March following rainfall during February (95 mm), but in lower numbers than the first year experiment. Seed set was less than in the previous year, due to continuing drought conditions (Figure 2). On average, 10 seedlings/m^2 emerged across the site (Figure 3). Similar to the previous year, the highest seedling number (112/m^2) was observed where the sward was uncut, extra seed had been added and the soil surface layer had been disturbed through scarification and raking. All emerged seedlings died 24 weeks after emergence.

Though substantially less wallaby grass seedlings emerged in the second year, seedling recruitment in the UC treatment was slightly higher than the CR treatment ($P<0.05$), in contrast to the previous year. Similar to the first year, the SA treatment had significantly more seedlings than the NS or IS treatments ($P<0.001$), while more seedlings were observed in the SR treatment than the NP or HA treatments ($P<0.001$). Within CR plots, seedling recruitment was slightly higher for the IS treatment than for SA and NS treatments ($P<0.01$); this was not observed in the first year. Across all treatments, the SA x SR combination generated the most number of seedlings ($P<0.01$). This repeated the results of the first year.

In general less bare ground (35-50 %) and more green plant material (~0.5 t/ha DM) maximised seedling numbers at emergence. This range was higher than the first year, possibly reflecting the drier seasonal conditions and greater sensitivity of seedlings to competition, leading to high death rates by early spring.

While the survival of seedlings in this experiment was poor, the general indication was that no modifications were needed to the sward structure, apart from light soil scarification at seed maturity. A small positive insecticide effect was also noted.

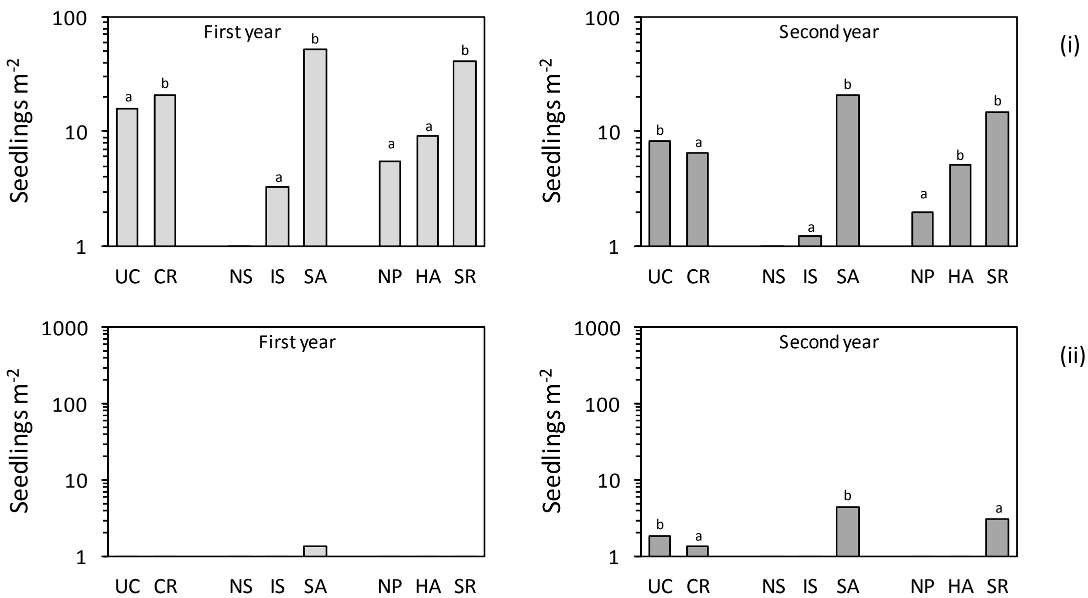


Figure 3. The average number of wallaby grass seedlings/m^2 (logarithmic scale, n + 1) in (i) March and (ii) 6 weeks after emergence, across UC (uncut), CR (cut and remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarification and raking) treatments for both first (2007-2008) and second (2008) year experiments. Results are not significantly different ($P<0.05$) where the same letter appears on a column within each subset of treatments in the same year.

4.2 Red grass experiment (Wellington)

Monthly rainfall and maximum and minimum temperatures at Wellington are shown in Figure 4 for the 2006-2008 experimental period, along with the previous 60-year averages.

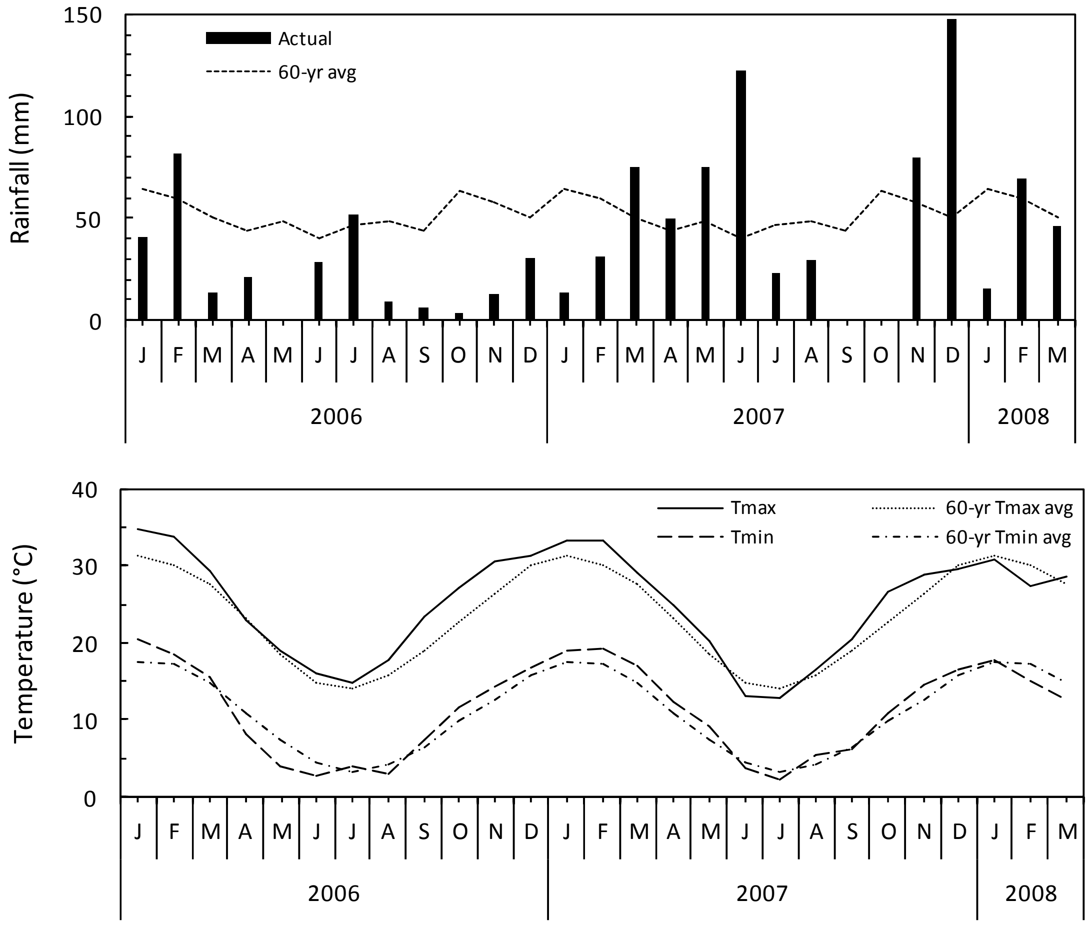


Figure 4. The monthly rainfall and maximum (Tmax) and minimum (Tmin) temperatures at Wellington for the 2006-2008 experimental period plotted with the 60-year averages (1946-2005)

Red grass seedlings emerged in early autumn of 2007 after rainfall events in late summer (February: 31 mm; Figure 4) and early autumn (March: 75 mm). The highest seedling number recorded was 279 seedlings/m^2, where the plot was pasture cropped, extra seed had been added and a low level of herbicide had been applied (Figure 5). Emerged red grass seedling numbers gradually declined and survival rates were very low. While an average of 24 seedlings/m^2 emerged at the site, this decreased to 7 seedlings/m^2 24 weeks after emergence. Growth conditions were tough through the summer and on average 1 plant/m^2 survived 52 weeks after emergence. The highest initial recruitment occurred on plots that had been pasture cropped, where extra seed had been added and where a low rate of herbicide had been applied. These plots also had the highest number of young plants (11/m^2) surviving after 24 weeks. These results clearly indicate that achieving a good initial seed set is important for satisfactory recruitment.

The increasing level of disturbance from ungrazed (UG) to grazed (GR) to pasture cropped (PC) treatments resulted in increasingly higher emergence of red grass seedlings. The PC treatment had substantially higher seedling numbers than the UG and GR treatments ($P<0.001$). Emergence of seedlings was significantly increased by seed addition (SA) ($P<0.001$), with few seedlings in the plots with no seed addition (NS). More seedlings emerged in plots with herbicide application (HA) than no herbicide application (NH), but this difference was not statistically significant. The addition of seeds did not have a significant effect within UG plots but increased seedling numbers significantly within GR and PC plots (P<0.001). The SA x PC combination produced the greatest seedling numbers (P<0.001). The ranking of treatment effects six weeks after emergence remained unchanged 24 weeks after emergence. By 52 weeks after emergence seedlings only survived in the PC x SA treatment combination.

Multiple regression analyses showed that maximum red grass seedling emergence was associated with litter DM of 1.4 to 2.2 t/ha, bare ground comprising 35-55% of the soil surface and plant cover of 45-55%. High seedling numbers were observed in the bare patches created through the pasture cropping treatment (PC) that had seed added. However, the lack of a significant relationship between seedling numbers and bare ground within the PC treatment suggests the addition of seed was more important then bare ground. Use of regression trees across all treatments showed bare ground as the most important factor determining initial seedling numbers (Figure 6). Where bare ground was less than 35% of the soil surface, average seedling numbers were 10/m^2 (n = 281), whereas more than 35% bare ground had on average 52 seedlings/m^2 (n = 151). This presumably reflected competition effects for moisture during the relatively dry conditions at the experimental site. At 24 weeks after emergence, highest plant survival occurred on areas where plant cover was 35% and bare ground comprised 10-15%. Regression tree analysis across all treatments, though, showed bare ground to be the most important factor (as was the case during initial emergence) in determining survival of young plants 24 weeks after emergence (Figure 7). Where there was more than 10% of bare ground an average of 14 plants/m^2 survived, whereas only 2 plants/m^2 survived where it was <10%.

The low soil moisture levels at the experimental site meant that more bare ground, in association with more litter, had a positive influence on recruitment. However, this may in part be a reflection of the pasture cropping treatment, which substantially increased recruitment. This suggests that no initial modification is needed to swards, apart from scarifying, to enable high recruitment rates. Sub-lethal doses of grass herbicide prior to seedling recruitment events also appear to be beneficial.

Flowering seed heads of wallaby grass (*Austrodanthonia* spp.), October 2007. (Photos: R. Thapa)

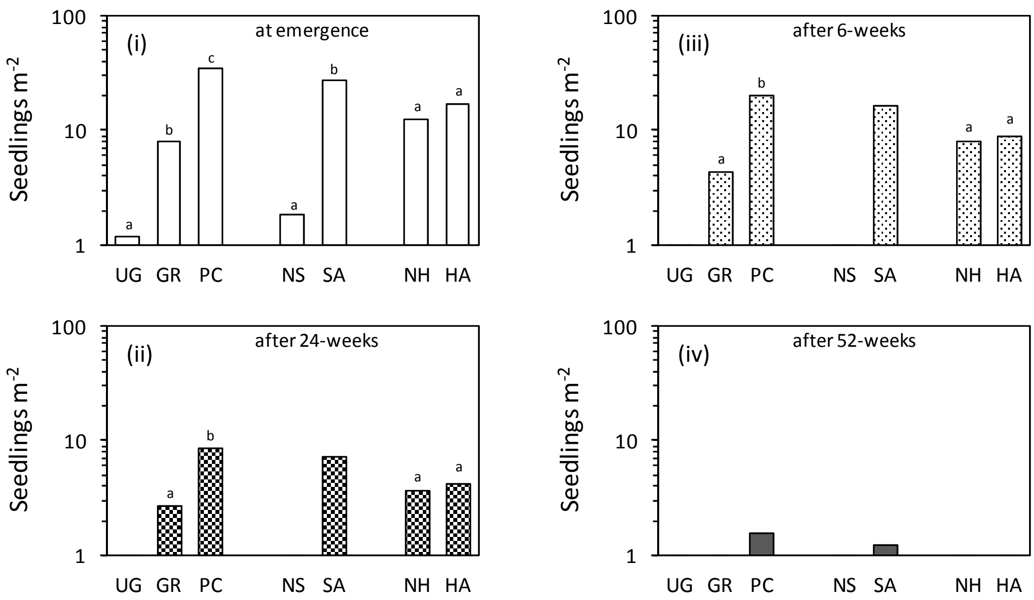


Figure 5. The average number of red grass seedlings/m^2 (logarithmic scale) in: (i) March; (ii) 6 weeks after emergence; (iii) 24 weeks after emergence; and (iv) 52 weeks after emergence across UG (ungrazed), GR (grazed), PC (pasture cropped), NS (no seed), SA (seed addition), NH (no herbicide), and HA (herbicide application) treatments. Results are not significantly different (P<0.05) where the same letter appears on a column within each subset of treatments in the same year.

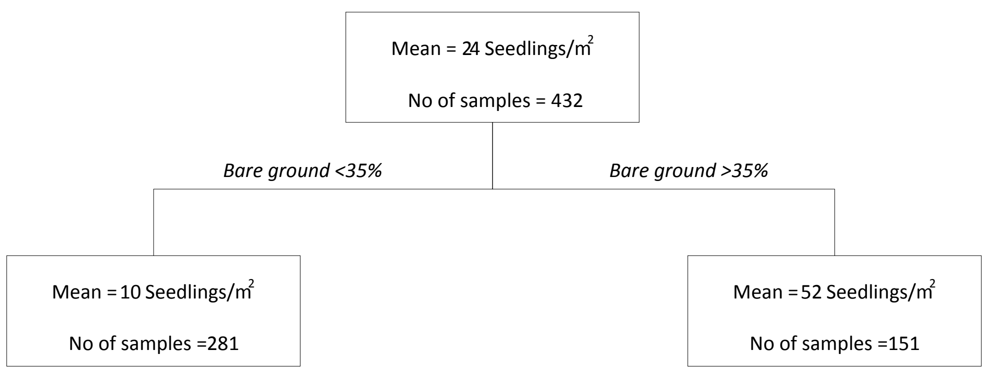


Figure 6. Initial red grass seedling emergence/m^2 in March 2007, as predicted by bare ground (%) across all treatments (standing DM, functional groups DM, green DM, litter DM, bare ground and plant cover were included in the model). Proportional reduction in error (PRE) = 0.105.

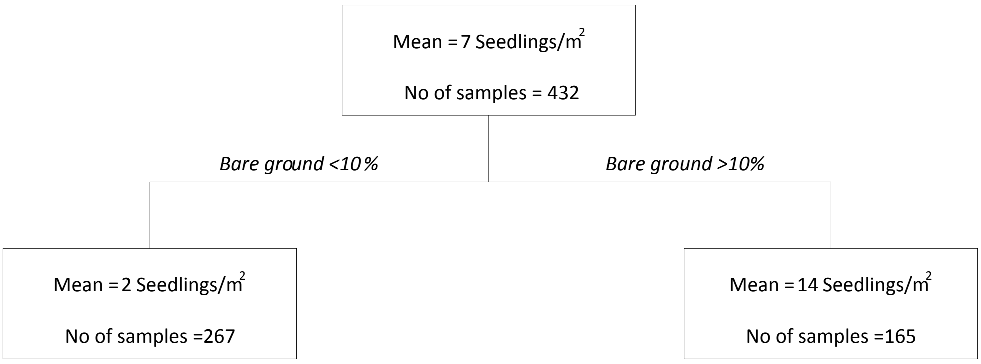


Figure 7. Surviving red grass young plants/m^2 at 24 weeks after emergence in August 2007, as predicted by bare ground (%) across all treatments (DM, functional groups DM, green DM, litter DM, bare ground and plant cover were included in the model). Proportional reduction in error (PRE) = 0.09.

4.3 Irrigation experiments

The number of days with no recruitment, but where the soil was moist from irrigation and with no further rain, averaged up to 6 days. Where some recruitment occurred from either irrigation, seed addition or rainfall, there was at least 10 days of moist soil. These treatments suggest that at least 7 days of moist soil is needed to obtain any seedling recruitment.

At Trunkey Creek 13 seedlings/m^2 were recorded in mid January 2007 when water was added without seed addition. This event coincided with a rainfall event that extended the period of moist topsoil to 10 days. A single recruitment event (2 seedlings/m^2) occurred in mid September 2007 as a result of water application in the preceding month. Limited recruitment (9 seedlings/m^2) in a seed addition treatment occurred in early March 2008 from a watering event in late February followed by rainfall shortly afterwards. The watering treatment in late February 2007 failed to generate any seedlings in early March when rainfall events that coincided with irrigation extended the period of soil moisture to 10 days. The general lack of recruitment in irrigated treatments reinforces the view that native grass recruitment depends predominately on seed availability, increasing the availability of micro-sites and adequate soil moisture conditions for germination and early seedling growth.

The irrigation treatments provided estimates of soil moisture conditions that were insufficient for seedling recruitment. Irrigation may have resulted in initiation of the germination process (priming of seeds), but without follow-up rainfall this did not lead to germination and seedling establishment. Estimates of soil moisture conditions showed that if there was no further rain, the top 50 mm remained moist for only ~5 days following irrigation of 50 mm over 2 days. When irrigation coincided with a rainfall event the soil remained moist for an average of 24 days and this resulted in some recruitment.

4.4 Soil moisture model

Soil moisture levels in the top 50 mm were estimated during successful recruitment events using the SGS model. The periods were identified when the soil moisture content was between field capacity (~40% v/v), where free water is available for seedlings to develop, and the permanent wilting point (~20% v/v), where there is little available moisture for developing seedlings. At both Trunkey Creek and Wellington there were two rainfall events in the February-March period of 2007 that raised soil moisture in the top 50 mm close to field capacity and preceded seedling recruitment events in March. It is apparent that either or both of these rainfall events were significant for seedling recruitment. The soil moisture model showed these two rainfall events were usually separated by a maximum of two days in which the soil was dry. Since sampling for recruitment observations was done after the second rainfall event and daily measurements of recruitment were not taken, it is not possible to say if there were any seedling deaths during these drier days. It is possible that the first rainfall event could have initiated the germination process (priming of seeds), with the second event resulting in germination and establishment. It is also possible that seedlings may have germinated from the first event and accessed moisture from a deeper layer. Further work is needed to evaluate the mechanism.

The recruitment events recorded across the experiments had reasonably common outcomes. Differences between the two species were neither apparent in timing of the recruitment event, nor in the general climatic conditions under which it occurred. Successful recruitment (Table 1) occurred when the top 50 mm of soil remained moist for at least 14 days, with no more than two days between rainfall events where the soil surface dried out. This period generally started in mid-February and extended to early March, when mature, germinable seed was present. Through those months there is often only one main rainfall event. Further analyses are needed, but the critical requirement appears to be the number of rain days above some threshold, rather than total rainfall *per se*. This study suggests that 8-14 mm per rain day, spread over seven rain days, during a total of 11 days is the minimum requirement for seedling establishment.

Successful recruitment occurred from the first significant rainfall event after seed maturation. A successful recruitment event could in practice occur through February until late March. After that period, a greater germination of competitive annual grasses could be anticipated. The parameters derived from these analyses then set the main boundaries to determine the frequency of achieving a successful recruitment event.

The climatic analysis could not be extended to the conditions required through the following summer for plant survival, due to low plant survival. Climate records over the months of December and January (Table 2) showed rainfall was below average in most cases and average maximum temperatures were around 24-30°C. Dry periods of 20-30 days still enabled some seedlings to survive, though only at low densities, as these summers were much drier than the long term averages (Figures 2 and 4).

Table 1. Rainfall events in February-March that kept the soil in the top 50 mm moist (~20-40%), resulting in seedling recruitment of wallaby grass at Trunkey Creek and red grass at Wellington. Soil moisture data was generated from the SGS pasture model (percent volume is the range in estimated soil moisture). Seedling recruitment is the mean for the whole experiment.

Recruitment event			**Rainfall**				**Soil moisture conditions**		
year	date	Seedlings per m^2	event	mm	date	rain days	% vol (0-5 cm)	date	moist days
Wallaby grass (Trunkey Creek)									
2007	13 Mar	24	1	35	18 - 21 Feb	4	38 - 20	18 - 23 Feb	6
			2	33	26 - 28 Feb	3	43 - 20	26 Feb - 2 Mar	5
			Total	*68*	*18 - 28 Feb*	*7*	*38 - 19*	*18 Feb - 2 Mar*	*11*
2008	10 Mar	10	1	59	1 - 8 Feb	3	44 - 23	1 - 9 Feb	9
			2	18	12 - 13 Feb	2	34 - 18	12 - 15 Feb	4
			Total	*77*	*1 - 13 Feb*	*5*	*44 - 18*	*1 - 15 Feb*	*13*
Red grass (Wellington)									
2007	14 Mar	25	1	32	26 Feb - 1 Mar	4	34 - 20	27 Feb - 4 Mar	6
			2	33	5 - 8 Mar	3	41 - 18	6 - 13 Mar	8
			Total	*65*	*26 Feb - 8 Mar*	*7*	*34 - 18*	*26 Feb - 13 Mar*	*14*

Table 2. Rainfall (mm), mean temperature, and the longest dry period over the December-January period (estimated by soil moisture in the top 50 mm) and average seedling survival 52 weeks after emergence of wallaby grass at Trunkey Creek and red grass at Wellington.

Site	**Month**	**Rainfall (mm)**	**Longest dry period**	**Mean temp (°C)**	**Survival at 52 weeks (seedlings/m^2)**
Trunkey Creek	Dec 07 Jan 08	35 39	28 Dec 07 – 18 Jan 08 (22 days)	30	1
Wellington	Dec 07 Jan 08	148 16	1 Jan -30 Jan 08 (30 days)	24	1

4.5 Past climate data and estimated frequency of recruitment

Climate data for the last 30 years at both study sites were used to determine the probability of successful recruitment events. The SGS model was used to determine the number of days of moist soil that resulted from each rainfall event, allowing up to two dry days between close rainfall events. Analyses were confined to the period between February 1 and March 31, when freshly produced seed should be mature. The aim was to identify the initial period after maturity when recruitment was possible.

The analyses showed that Trunkey Creek had an average of 14 days each year that would keep the soil surface moist following a significant rainfall event in the February-March period, while it was 12 days for Wellington (summarised in Figure 8). This implies that some recruitment is possible in most years at both sites. The shortest moist period was two days at both Trunkey Creek and Wellington, which would be insufficient for a recruitment event, while the longest moist period at both sites was 24 days.

Further analyses showed that Trunkey Creek would only fail in 8% of years to achieve the minimum moisture conditions of seven days, while there was a high chance of recruitment in 44% of years (Figure 8; Table 3). At Wellington the analyses suggested minimum moisture conditions of seven days would not be achieved in 28% of years, while there was a high chance of recruitment in 30% of years (Figure 8; Table 3).

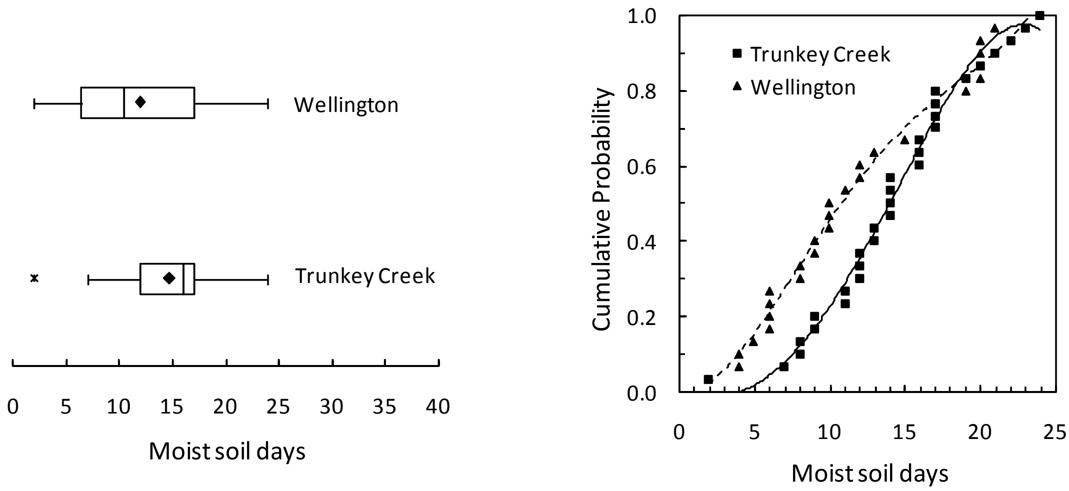


Figure 8. Number of days over the last 30 years at Trunkey Creek and Wellington when the soil was moist in the top 50 mm after a significant rainfall event in the February-March period, shown as box plots (left-hand side), with average values (as diamonds) and outliers (as asterisks), and their probability distributions (right-hand side).

Analysis of the longer-term data showed that the average rainfall coinciding with the median days of soil remaining moist for 14 days was 44 mm and 47 mm for Trunkey Creek and Wellington, respectively (Figure 9; Table 3), though in each case there was considerable variation. These values are less than those identified from the experiments of 57-99 mm. This reinforces the point that the number of days of moist soil above a threshold is the most important criterion that determines the success or failure of a recruitment event.

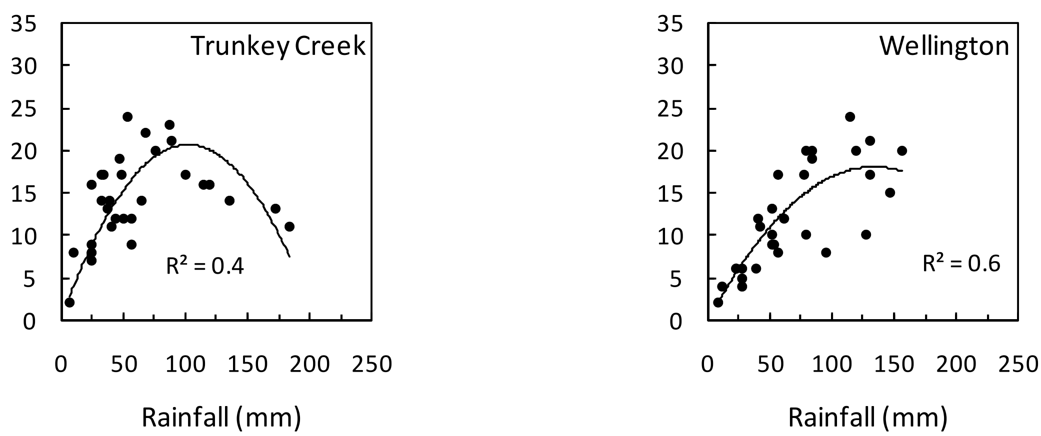


Figure 9. Rainfall events in the February-March period and the corresponding estimated number of days of moist soil in the top 50 mm due to that particular event over the last 30 years at Trunkey Creek and Wellington. Quadratic curves were fitted to the data to show trends.

Table 3. Median days of moisture for recruitment in the top 50 mm of soil and the probability of exceeding the minimum and optimal soil moisture conditions of 7 and 15 days, respectively and the average rainfall coinciding with the median, 7 and 15 days of moist soil over the last 30 years at Trunkey Creek and Wellington. Estimates are derived from probability distributions in Figure 8 and rainfall versus moist soil days in Figure 9.

Site	Median (days)	Probability of exceeding (%)		Rainfall at (mm)		
		7 days	15 days	median days	7 days	15 days
Trunkey Creek	14.0	92	44	44	20	48
Wellington	10.5	72	30	47	30	80

Estimates over the last 30 years of the first significant rainfall event after seed maturation (from 1 February) that would keep the soil sufficiently moist in the top 50 mm for a recruitment event, showed this typically occurred at Trunkey Creek and Wellington by late February (Figure 10). In some years the first significant rainfall of the year did not occur until later in March. Such events could still prove successful for recruitment, as temperatures at this time are still close to 20^{o}C, though more competition from annual grasses could be expected.

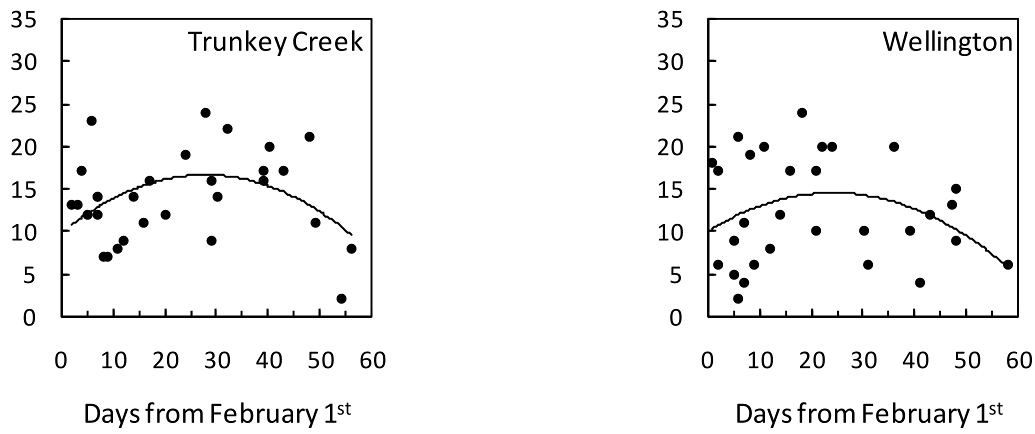


Figure 10. The first significant rainfall event after seed maturity starting from 1 February (denoted as rainfall start day 1) until 31 March (day 59) against the corresponding number of days of moist soil in the top 50 mm due to that particular event over the last 30 years at Trunkey Creek and Wellington. Quadratic curves were fitted to show trends.

The climate analyses over 30 years support the experiment results that there is a reasonable probability of an adequate rainfall event for successful recruitment of wallaby grass and red grass each year in central New South Wales.

5. Discussion

Previous studies of seedling recruitment of sown temperate perennial grasses (Lodge, 1981; Dowling, *et al*., 1996b; Waller *et al*., 1999; Virgona and Bowcher, 2000; Lodge, 2002a; 2004) have suggested that successful recruitment events are rare. However, the results of these experiments indicate that enabling seedling recruitment of desirable native perennial grasses within existing swards is a viable practice that farmers can employ. Despite drought conditions, seedlings successfully established at both sites in each year of study. Some seedlings survived through to the next autumn, though the sward conditions resulting in enhanced seedling survival through the first year were not investigated.

5.1 Principles

The results for both wallaby grass and red grass indicate that removing stock from paddocks (resting) to maximise flowering and seed set creates sward conditions that can lead to higher recruitment rates. This suggests that vertical litter may be more useful than 'cut and leave' treatments that retain the same biomass, but place the 'litter' in a horizontal position. It is possible that vertical litter increases the boundary layer resistance that reduces evaporative demand and lowers moisture stress on emerging seedlings. This effect is less with horizontal litter, although a 3-dimensional sward structure may be important for enhanced recruitment.

Higher seedling numbers were often associated with more herbage mass and plant cover, except in drier conditions where competition for moisture was more intense and more bare ground proved important. Under dry conditions soil scarification proved beneficial by increasing the amount of bare ground. This was important on soils prone to having gleyed surfaces, but less so on self-mulching soils. In practice this could be done on farms with harrows or a light scarifying implement dragged behind a 4WD vehicle.

Insecticide and herbicide treatments had some benefits, although their effects were variable and not well defined. Insecticides could be beneficial when ant activity is intense and seed set is sub-optimal, such as may occur in dry years or where some grazing of seed heads has occurred. In wet years seed production is likely to exceed the capacity of ants to remove them all and as a result they will be of less concern.

Low rates of grass-specific herbicides, applied at seed maturity to lessen competition from mature plants and annual grass seedlings, generally had small effects. This was unexpected and may have been due to the constraint of dry seasonal conditions on the competitiveness of other grasses. In these experiments annual grasses were anticipated as a problem, but in the event were only a minor component of each pasture. However, it is known that annual grasses are often a serious competitive problem for perennial grass seedlings. Annual grasses tend to have larger seedlings and produce higher seedling densities than perennial species. The sub-lethal grass-selective herbicide treatment employed in this research showed that grass herbicides can be successfully used without damaging mature plants of desirable perennial grass species, and without adverse effects on subsequent germination and recruitment of seedlings. In most seasons, however, the main recruitment event for perennial grasses is likely to occur before germination of annual grasses, suggesting the need for grass herbicide applications is not likely to be common. Herbicides for broadleaf weed control can be used if a significant post-emergence weed problem emerges, without risk of damage to the grasses. In much of the landscape where native grasses occur, competition from weeds at the time of perennial grass recruitment may not be a major problem in most years.

The rainfall events that triggered successful recruitment in these experiments typically occurred in late summer - early autumn and resulted in ~15 days of moisture in the top 50 mm of soil. These events were successful, even when there were two dry days during this period of soil moisture. A moist soil period of <7 days was not adequate for successful recruitment. Seedlings may have emerged from those events, but none were detected when counts were taken 2-3 weeks after the rainfall events. The modelling work indicates a high probability of obtaining a 7-day moist soil event around the appropriate time in late summer- autumn at these sites, while a 15-

day moist soil event is of lower probability. The Trunkey Creek tablelands site would expect some recruitment in most years, while the lower rainfall Wellington site would still have a 30% chance of ideal soil moisture conditions for recruitment. The median moist soil days at Wellington of ~10 days suggests some recruitment in at least 50% of years.

5.2 Seed dormancy and seed banks?

The field experiments showed one major recruitment event occurred each year at both sites. Observations of those experiments through the year failed to identify any other significant germination events. The irrigation experiment similarly found few seedlings emerging at other times. This result is perplexing. The soil cores taken through the year and tested for readily germinable seeds, found low emergence of perennial grass seedlings outside the critical February-March period. Other studies (King *et al.*, 2006) at the Carcoar SGS site in the central tablelands of NSW found almost no perennial grass seedlings emerged from cores kept in a glasshouse for a year. Previous research has shown that seed banks of perennial grasses are usually at low levels in the soil (Virgona and Bowcher, 1998; Lodge, 2001; 2004), even though the amount of seed produced is often high (Lodge, 2004; Kelman and Culvenor, 2007). Several other studies (Winkworth, 1971; Eberlein, 1987; Chambers, 1989; Silcock and Smith, 1990; Silcock *et al*, 1990; Anderson *et al*., 1996) claim the seed of many grass species remains viable in the soil for as little as two years. These collective results all support the view that natural recruitment rates are likely to be low outside the 'window of opportunity' found around February - March, soon after seed maturation.

The role of dormancy in the native grasses is unclear, though tests of these species showed that a high proportion of the seeds were dormant (J Stevens, unpublished data). A general conclusion is that there is only one main recruitment event for these species each year, that being the first significant rainfall event after seed maturation. If a large proportion of that seed is dormant, recruitment will often be seed-limited and all other factors need to be optimal to maximise recruitment. Thus, current season seed set is critical and little reliance can be placed on soil seed banks.

5.3 Where will the technology work?

Enabling the recruitment of perennial grasses should be possible in regions of south-eastern Australia that are characterised by occasional, but significant, rainfall events in late summer-autumn. Often after an event that enabled seedling recruitment, little rain fell for the subsequent 2-3 months, yet a reasonable number of seedlings survived, indicating that continuing rainfall through autumn is not essential. If a high number of seedlings initially establish enough are likely to survive through to spring to achieve the goal of increased perennial grass densities. The fact that wallaby grass and red grass occur naturally across the higher rainfall zones of south-eastern Australia suggest these methods have general application for encouraging perennial grass recruitment. The key climatic requirement appears to be rainfall when soil temperatures are at least 20°C, which results in rapid seedling growth of the perennial grasses and low germination of annual grass weeds. If temperatures are lower, a longer period of moist soil is likely to be required to enable recruitment, due to slower root development. However, further work is needed to clarify the applicability of these results to other regions and to determine whether the same principles apply to other important native perennial grasses, such as *Themeda australis* and *Microlaena stipoides*.

5.4 Development of a decision support chart

Based on the general results from the experimental sites at Trunkey Creek and Wellington, a decision support chart (Figure 11) was developed to summarise the main recommendations from this study. The objective of this tool is to provide farmers and their advisors with a simple pasture management strategy that enables them to encourage recruitment of desirable perennial grasses.

The starting step is to select a paddock in spring with sub-optimal perennial grass content. When first using the techniques, paddocks should be selected where the perennial grass content is around 30% and without major weed problems. This will enable farmers to gain experience and see results in a reasonable time. Where major weed problems occur, they need to be controlled to some extent before implementing this strategy. If perennial grass content is less than 20%, the opportunity to readily enhance perennial grass content is lower and other actions may be needed to bring the paddock to a point before these recommendations can be implemented. Weed control, in particular, may be needed to ensure that any seed produced is mostly from the desirable grass species.

The selected paddock should be locked up with stock removed by late spring to allow the grasses to flower and set seed and for that seed to mature and fall (*i.e.* a summer rest). Seed banks of perennial grasses are usually at low levels in the soil (Virgona and Bowcher, 1998; Lodge, 2001; 2004), which emphasises the need to promote flowering and seed set. Assume that recruitment will depend predominately on current seed set.

If the paddock has a history of annual grass and broadleaf weeds, control measures need to be used. A sub-lethal selective grass herbicide treatment can be used on desirable perennial grasses, with no deleterious effect on seedling recruitment. Broadleaf weeds can be readily controlled without posing problems for perennial grass recruitment. In much of the landscape where native grasses are widespread, these short-term weed problems will only be occasional. The more serious problem, particularly in central NSW, is serrated tussock (*Nassella trichotoma*), which will out-compete new perennial grass seedlings. Current recommendations for its control are to spot-spray plants, minimising collateral damage to desirable species.

Seasonal conditions need to be carefully monitored through December and January to decide if the paddock rest should be continued, if any scarification or insecticide treatments are needed, or if there is unlikely to be any useful recruitment because of a dry season.

Results from Wellington, where only 40% of average rainfall was recorded for the period December 2006-January 2007, demonstrated how low rainfall limits plant growth and seed production. It is suggested that unless 40% of average rainfall is received, the chances of obtaining a good seed set will be low. If the season is poor (<40% average rainfall) the best decision is to forego the option of fostering recruitment, as flowering and seed set will be limited, the chance of sufficient rain at the right time for a recruitment event is reduced and any remaining feed will be needed for livestock.

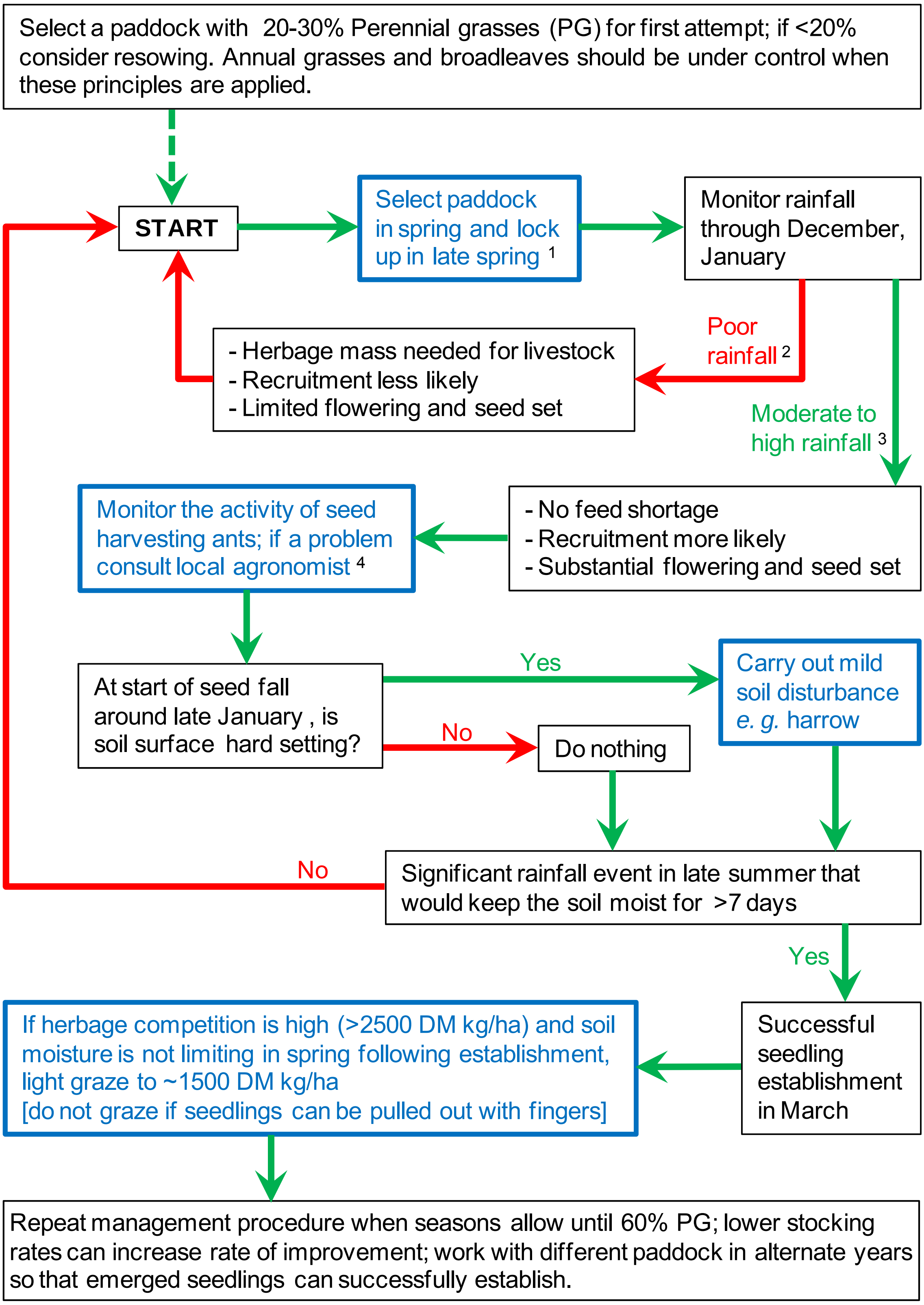
If seed harvesting ants are very active and seed set is low, applying an insecticide before seed fall may be beneficial. However, the benefits of using insecticides as a general rule on native grasses do not seem large enough to justify the cost.

The presence around late January of suitable micro-sites for mature seeds to lodge is essential and depends on the soil characteristics of the paddock. On most soils a light scarification can enhance recruitment and in a dry season this has the added advantage of reducing competition from existing plants. On self-mulching soils scarification is not required.

Bare ground appears to be one of the most important requirements for seedling recruitment of native grasses. A bare ground proportion of 30-50% offered the greatest advantage, particularly in the dry seasons experienced in these experiments. A low level of litter enhanced recruitment and there appeared to be an upper limit of ~1-2 t/ha DM, above which recruitment declined. A combination of these factors provided the most favourable micro-site conditions for seedling emergence.

The analysis of climatic conditions showed that a significant rainfall event in late February or March resulted in adequate soil moisture for seedlings to establish. The conditions for maximum seedling recruitment appear to be 15 days of moist soil in the top 50 mm, with a maximum of two dry days during this period, while the minimum condition for any recruitment appears to be at least seven days of moist soil. These analyses suggest that in most years some recruitment will occur and that if possible, farmers should rest paddocks from spring through to late March, if seasonal conditions permit, to maximise the opportunities for recruitment.

Although the conditions for survival of young plants were not resolved in this study, general observations suggest competition from existing species may be the biggest factor during early growth. Light grazing from 12 weeks after emergence is an option once seedlings become robust. The subsequent survival of young plants may depend more on adequate soil moisture conditions being maintained and limited competition from other plants through summer. General guidelines for managing sown pastures are likely to be applicable to managing pastures following natural recruitment at this stage of our understanding of these ecosystems.



Notes:

[1] If dry spring, forego the option of fostering recruitment as flowering and seed set will be limited over summer

[2] Poor rainfall equates to less than 40% of average rainfall for December-January

[3] Moderate rainfall equates to at least 40% of average rainfall for December-January

[4] Ants are of the most concern with phalaris while they are less of a problem with native species

Colour codes: Green = Moving forward; Red = Exit points; Blue = Actions to be done in the field

Figure 11. Decision support pathways to enable recruitment of perennial grasses

6. A management package for recruitment of desirable perennial grass seedlings

The following advisory package has been developed to provide farmers and their advisors with a simple low-cost pasture management strategy to encourage emergence of new seedlings (*i.e.* seedling recruitment) of desirable perennial grasses within existing pastures in the 600 - 800 mm rainfall zone of south-eastern Australia. Although it is mainly aimed at native pastures containing desirable perennial grasses, the principles could also be applied to sown perennial grass pastures.

6.1 Principles

Allowing new seedlings of desirable perennial grasses to establish within existing swards is a viable practice that farmers can use to restore pastures to a more productive state (~60% desirable perennial grasses), without the need for re-sowing. The investment costs are considerably less and restored paddocks are more profitable over time.

Work with one or two paddocks at a time, rather than the whole farm, in the first instance. Start by selecting a paddock with a moderate content of desirable perennial grasses (around 20-30% is ideal), rather than the poorest paddock. Better paddocks provide faster rates of improvement and quick seed production. The seeds are then available for harvesting and redistribution to poor paddocks.

The paddock has to be free of major weed problems. Control annual grasses and seasonal weeds, with herbicides and grazing, in the year before pasture improvement is attempted. Desirable perennial grasses can tolerate low rates of herbicide applications. Having a paddock relatively free of weeds is a prerequisite for success of this management strategy.

Few seeds of perennial grasses generally remain in the soil from previous seasons. Therefore, emergence of new seedlings depends predominately on seeds set in the current year. Grazing needs to be managed to encourage flowering and seed set.

Rest paddocks (i.e. remove stock) to maximise flowering and seed set creates sward conditions that enhances emergence of seedlings. Standing plant material (even if ~80% dead) is more useful than litter (dead plant material lying on the soil surface) in most instances. More than 2000 DM kg/ha of litter is detrimental to emerging seedlings.

Determine basic soil characteristics of the paddock, including nutrient status, pH and texture. Fertilisers should be applied to address nutrient deficiencies that affect seedling growth, especially in low fertility areas. Phosphorus, in particular, is important for early root growth of seedlings. Roughing up the soil surface by harrowing, especially on soils that are hard setting, increases the locations where seeds can lodge and germinate, thus enhancing emergence of new seedlings.

Consider use of an insecticide to control seed harvesting ants if they are a problem. This is often the case with phalaris in sown pastures.

Rainfall events in late summer or early autumn that result in at least 10 days of moist soil in the top 50 mm triggers successful seedling emergence -15 days of surface soil moisture is ideal. Seedling emergence is not hindered by 2 days where the soil surface is dry during this period.

Some establishment of new seedlings will occur in most years, but resting paddocks from late spring through to late March and following the management strategies in this bulletin will maximise the opportunities for seedling emergence.

In the first year of applying these management tactics, a conservative rate of improvement in perennial grass content may be 0-20% of the total herbage mass produced per year. It could take 4-5 years to reach a target of 60% desirable perennial grasses, if there is an average of 10% improvement per year.

6.2 Seasonal management tactics

Spring

Do not let the selected paddock get overgrown in early spring. Aim to have the herbage mass around 1500-2000 kg/ha of dry matter when the paddock is locked up in late spring to allow plants to flower and set seed.

Early – mid summer

Allow seeds to mature and fall onto the soil surface. Monitor seasonal conditions in December and January. Continue the summer rest if rainfall for these months is >40% of average rainfall.

If the season is poor (<40% average rainfall in these months), defer setting up the paddock for seedling establishment until the next year. Even if rains in late February provide the right conditions for recruitment, seed set will have been minimal and feed will be needed for livestock.

Consider applying an insecticide if seed harvesting ants are present in high numbers.

On hard-setting soils carry out mild soil disturbance to provide micro-environments for seeds to lodge and germinate. This can be done using harrows dragged behind a vehicle.

Late summer - early autumn

Look for new perennial grass seedlings within 2-3 weeks of a significant rainfall event in late February or March.

Autumn - winter

Once seedlings cannot be pulled out easily by hand, the paddock can be lightly grazed until ~1500 kg/ha of dry matter remains.

Guidelines developed for managing sown pastures are also applicable to managing pastures enhanced through natural establishment.

6.3 Benefits

Costs involved in improving paddocks through natural seedling recruitment will be substantially less than re-sowing a new pasture, as few inputs are required. In a good season resting one or two paddocks will have little impact on the feed supply needed to carry livestock on the farm. Soil disturbance using harrows may cost ~$20/ha and is only necessary for hard-setting soils. Insecticides and herbicides will incur some additional costs if they are needed. However, this will be considerably less than the ~$300/ha needed to sow a new pasture.

Once a stable target of 60% content of desirable perennial grasses is reached, increased livestock production will result, either through greater carrying capacity or more production per head. Estimates for some native grass pastures in New South Wales suggest livestock production can be more than doubled in the medium term, depending on soil characteristics.

It is useful for farmers to rest some paddocks to enable seedling establishment if suitable seasonal conditions prevail. If the conditions turn dry over summer then emergence of new seedlings is less likely. Rested paddocks can then be grazed, as feed is likely to be limited at this time. In wet summers there is usually no shortage of feed and resting paddocks for longer periods is not likely to cause major difficulties.

7. Impact on the Livestock Industries

Surveys done over recent years (Kemp and Dowling, 1991) found that the perennial grass content of pastures surveyed was often only 20%, when those species were present. Research done at the Carcoar SGS site (Michalk *et al.* 2003) indicated that at least 60% perennial grass is needed to have useful impacts on increasing livestock production, reducing weed outbreaks and the development of soil acidity and better utilising soil water to minimise salinity problems. Earlier studies (Kemp *et al.,* 1996) demonstrated that following summer rests the proportion of cocksfoot (*Dactylis glomerata*) in a pasture increased from 10% to 50% over four years. That work was unable to provide an insight into the mechanisms involved, though observations indicated new plants had established. This background, when considered with the research presented in this project, suggests that a steady improvement in the perennial grass content of typical pastures across south-eastern Australia is now possible in a predictable manner.

In recent times farmers have sown few new pastures and reduced inputs, such as fertiliser, to their existing ones. The long-term effect is a decline in carrying capacity. However, the research in this project has shown that pastures can be restored to a more productive state and that the core issue of increasing perennial grass content can be managed. This is a critical first step in pasture improvement, as demonstrated at the SGS Carcoar site (Michalk *et al.,* 2003). That work showed that improving botanical composition of the pasture needs to precede other inputs, such as fertiliser. Farmers now have a low-cost approach to pasture improvement for those large parts of the landscape where there are still some desirable perennial grasses and no over-riding weed problems. The restored pasture may not be as productive as a newly sown pasture, but the investment costs are considerably less and hence, restored paddocks could prove more profitable.

A conservative rate of improvement in perennial grass content may be 10% *p.a.* (of the total herbage mass), such that from an average starting point of 20%, it could take four years to reach a target of 60%. Costs involved could be as low as nil, such as the case where a paddock is rested without any impact on forage availability for livestock. Costs will be progressively higher, where increasing levels of inputs are required, such as light scarification, insecticides for ant-control and herbicides for weed control. Of the practices identified, scarification appears to provide the most benefit, by creating micro-sites to improve recruitment rates, while the need for insecticides and herbicides is likely to be less common. A light scarification may only cost $20/ha. This is in contrast to ~$300/ha for sowing a new pasture.

Initially carrying capacity of a paddock may not change greatly during the early period of fostering increased native grass recruitment. But once the perennial grass content target of 60% has been reached then either higher stocking rates or more production per head will result. On many of the poor native grass areas in NSW it is not unreasonable to anticipate doubling production in the medium term.

Adoption of rotational grazing practices on perennial pastures has increased over the past 20 years or more (Kemp and Michalk, 1993; Kemp *et al.*, 1996). However, many farmers are unsure of the best grazing practices to help rehabilitate less productive pastures. This research provides solutions to improve the proportion of desirable perennial grass species in relatively unproductive pastures. In five years time it is anticipated that more farmers will be targeting paddocks for rest periods over summer to allow seed set of desirable perennial grass species, and will have a better understanding of the methods to improve pasture productivity.

8. Conclusions and Recommendations

This research showed that recruitment of native perennial grasses occurred in the field, that minimal intervention was required (rests to maximise seed set, light scarification on non-self-mulching soils and ant control and herbicide treatments in occasional circumstances) and that often only one rainfall event in late summer was needed to produce enough seedlings for a reasonable number to survive through to the next spring.

Key messages from this work are:

- The results were similar for both the C3 wallaby grass and the C4 red grass and common principles were established to enable successful seedling recruitment.
- Current seed set is critical for successful recruitment as there was limited germinable seed in the soil seed bank. Rest paddocks from spring to maximise flowering, seed set and maturity.
- Seed production is low during dry years and hence the numbers of seedlings establishing through those years will be less than could be achieved if extra seed was supplied, but still useful for increasing the perennial grass content if there is sufficient rainfall over the following year.
- Seedling recruitment was greater where there was:
    - more seed set (rest paddocks from spring),
    - more (uncut or ungrazed) herbage mass was present in intact swards,
    - where some soil disturbance (light scarifying) on non self-mulching soils occurred,
    - insecticide to control seed-harvesting ants (if a problem), and
    - herbicide at low rates at seed maturity to weaken plant competition i.e. before recruitment, may help reduce competition in some cases (this would apply if significant weed problems were evident or highly likely).
- The surface soil (top 50 mm) needs to be moist for > 7 days and ideally 15 days to enable a high density of perennial grass seedlings to establish. In all five experiments suitable conditions occurred around late February and through March each year, despite the dry seasons. These soil conditions resulted from 50-80 mm (more rain needed at the warmer site at Wellington) of rain over several days. Periods of 2 dry surface soil days in a 15 day period did not seem to limit recruitment.
- Analysis of the last 30 years of rainfall at each site found that the minimal requirement of 7 days of adequate surface soil moisture occurred in late summer, early autumn in 92% and 72% of years at Trunkey Creek and Wellington, while 15 adequate soil surface moist days occurred in 44% and 30% of years, respectively. This indicates that useful recruitment would occur in most years.

- Conditions for seedling survival through the following year were not resolved due to the drought. Future work needs to investigate the interaction between plant competition and soil moisture conditions on seedling survival within existing swards.

This work showed that in most years it would be useful for farmers to rest paddocks to enable recruitment if suitable seasonal conditions then followed. Typically the forage for grazing only becomes restrictive to livestock by late summer and autumn. Thus in dry years seedling recruitment and survival is less likely but a rested paddock has then some forage available that can be used. In wet seasons there is usually no shortage of fodder and resting the paddock for longer periods is not a major difficulty. The paddock would be out of use for less time than applies if sown to a pasture. After locking up paddocks they need to be monitored to decide if intervention is needed for scarification or application of insecticide or herbicide treatments. Costs are likely to be less than 10% that of sowing a new pasture. A decision chart and a set of management guidelines have been developed to guide farmers and their advisors to use the information gained in this project.

A consideration of climates suggests that the principles developed in this project will apply to native perennial grass pastures in many areas throughout south-eastern Australia, where significant rainfall events in late summer and autumn commonly occur. Some preliminary work would, however, be needed in different districts to determine if the management guidelines developed in this project need any modification.

Farmers are now in a position to more reliably enable recruitment of desirable perennial grasses within their pastures. The outcome of 60% perennial grass can be achieved at a significantly lower-cost than re-sowing exotic species and improved production and environmental outcomes can be achieved.

This work has identified key management practices that can be used, but there are several areas that need to be considered in future work. These include:

- Wider climatic analyses to resolve the regions across south-eastern Australia where the tactics developed in this project can be used. This would involve analyses of likely soil moisture conditions at the time of the year when recruitment is possible.
- Determining the importance of seed dormancy for native grass recruitment. It may be that dormancy is limiting the number of viable seeds available for recruitment and that predation removes many of those seeds before they can germinate. Field work is needed to investigate the use of dormancy breaking chemicals to maximise recruitment rates.
- Developing practices to maximise seedling survival through the year after emergence. This would require field work on the level of plant competition from desirable species and weeds, and studies to determine the best grazing strategies.

9. Acknowledgements

Roshan Thapa conducted much of this work through a PhD project funded by Meat & Livestock Australia. This work also formed part of a project through the Future Farm Industries CRC, with additional funding provided by Meat & Livestock Australia, Australian Wool Innovation Ltd and Land & Water Australia.

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Establishment of tedera (*Bituminaria bituminosa* var. *albomarginata*)

Future Farm Industries CRC Technical Bulletin

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Abstract

Tedera (*Bituminaria bituminosa* var. *albomarginata*) is a perennial legume from the Canary Islands of Spain that is undergoing intensive research through the Future Farm Industries Cooperative Research Centre (FFI CRC) as a potential new pasture legume for southern Australia. There is a paucity of research on all aspects of tedera, although it is well adapted to Mediterranean-type climates and can survive in environments that receive as little as 150 mm annual rainfall. Little, in particular, is known about the germination and establishment requirements of this species.

This research was undertaken to investigate factors influencing germination and establishment of tedera. It was conducted by Mr Cale Beard as an Honours project through Curtin University of Technology. The aim of this project was to study the germination characteristics of tedera, to gain an understanding of its seed biology and germination requirements, in order to determine optimum sowing conditions and identify any potential constraints to its establishment. Such information is crucial to enable this new species to be sown commercially.

The project was successful in developing a preliminary establishment package for tedera for testing in the field. The research used to develop these guidelines was conducted as part of an Honours project “Germination ecology of Bituminaria bituminosa var. albo-marginata and its suitability to the Mediterranean-type climate of Western Australia” through Curtin University and co-supervision by Kings Park and DAFWA (Beard 2009).

The key finding from this research is that there do not appear to be any major barriers to successful germination and establishment of tedera in the field, provided soil moisture conditions are adequate. An establishment package for tedera has been developed, on the basis of experimental results from this project and from common agronomic practices used for other crops and pastures.

Two tedera accessions were used. They were found to be relatively similar in regard to seed characteristics. Tedera seeds possess a non-deep physiological dormancy characteristic that restricts protrusion of the radical through the seed testa. Manipulation of the seed coat, by surgical cutting, scarification or threshing, was relieved this restriction and increased germination percentages by up to 72%. Tedera was found to possess after-ripening seed dormancy of three months from maturation, but this is not likely to cause problems for sowing, as seed harvested in early summer will not have dormancy issues if planted in early winter.

Tedera germinates readily in temperatures ranging from 5°C to 35°C, with optimum germination at 15-25°C. This implies tedera is well suited to germination in the autumn-winter period of southern Australia. Tedera had no germination requirement for light at 15°C and 20°C. Tedera germinated more readily under moisture stress than lucerne, but its germination did not differ from lucerne at any salinity level.

Tedera has a relatively large seed. A sowing depth trial showed that tedera seedlings readily emerge when sown from a depth of 2- 8 cm. The majority of seeds also emerged from 10 cm, the deepest seed placement tested, although there was a delay in cotyledon emergence. However, sowing seeds on the surface caused high mortality, with a low seedling emergence resulting. This result implies that conventional seeding equipment can be used to sow tedera and that it will emerge at high rates from a seeding depth ranging from 2-8 cm.

Priming of tedera seeds with the plant signalling compounds, smoke water, KAR1, and GA3 and priming with water did not produce higher germination percentages than un-primed controls, bit smoke water and KAR1 did increase germination rates. KNO3, on the other hand, inhibited germination, while ethylene produced different results between accessions. These effects need to be tested in the field, but the

ready germination and lack of major dormancy issues in tedera, suggest chemical priming is unlikely to be beneficial.

Contents

1. Background

Tedera (*Bituminaria bituminosa* var. *albomarginata*), is a perennial legume from the Canary Islands of Spain, particularly Lanzarote and Tenerife, that is undergoing intensive research through the Future Farm Industries Cooperative Research Centre (FFI CRC) as a potential new pasture legume for southern Australia. There is a paucity of research on all aspects of tedera, although it is well adapted to Mediterranean-type climates and can survive in environments that receive as little as 150 mm annual rainfall. However, little is known about the germination and establishment requirements of this species. Research into its seed biology is important to understand its germination requirements and to determine whether there are likely to be any impediments to its establishment in the field. This information is crucial to enable this new species to be sown commercially.

1.1 *Bituminaria bituminosa* var. *albomarginata*

1.1.1 Origin and distribution

Bituminaria bituminosa (L.) Stirt. is a perennial legume species with a large geographical distribution throughout the Mediterranean basin and Macronesian islands (Correal *et al.* 2008). There are three botanical varieties. The var. *albomarginata* is restricted to the Canary Islands of Spain, particularly Lanzarote and Tenerife. It is known locally as “tedera” and is found in a range of habitats, including coastal areas on the semi-arid island of Lanzarote with annual rainfall as low as 150 mm (Correal *et al.* 2008). The other varieties of *Bituminaria bituminosa*, var. *bituminosa* and var. *crassiucula*, are more widely distributed throughout the Mediterranean region. On the Canary Islands, both are present, with var. *bituminosa* being found in areas with 300-1000 mm annual rainfall and var. *crassiucula* being found in the high elevation subhumid area of Tenerife that receives 1700-2200 mm of annual rainfall.

Most types of var. *bituminosa* and var. *crassiucula* are not suitable for animal production, as they have low palatability, due to the strong odour the plant emits through oil glands in their foliage; this gives rise to the botanical name of *Bituminaria* for the species (Pecetti *et al.* 2007). However, var. *albomarginata* (tedera) types do not have a strong odour. Tedera has good palatability and is widely used in the Canary Islands as a forage for grazing livestock (Pecetti *et al.* 2007). This suggests its potential use as a perennial fodder species in southern Australian farming systems.

1.1.2 Flowering and seed production

Bituminaria bituminosa is a self-pollinating plant that develops one seed per pod (Correal *et al.* 2008). The seed is oval shaped with a large beak on one end (Fig. 1). Seeds are much larger than most other pasture legumes. A study by Correal *et al.* (2008) found the mean seed weight of variety *albomarginata* was 24.7 mg. This compares with typical seed weights for lucerne of ~2 mg and subterranean clover of 6-9 mg.

Tedera is a prolific seed producer. The study of Correal *et al.* (2008) showed that var. *albomarginata* has the highest mean seed production of the three varieties. Under ideal conditions individual tedera plants produced ~ 2,500 flower heads, and each flower head had 28 florets. Around 56% of the flowers produced a pod 30 days after flowering, so that individual plants produced up to 40,000 seeds (Correal *et al.* 2008). However, in order for tedera to be successfully introduced into Australian agriculture, an efficient seed production method needs to be developed. This is the subject of ongoing research in the Future Farm Industries CRC.

Figure 1. Seed of tedera.

1.1.3 Production and environmental benefits

Tedera is a perennial legume suited to the Mediterranean-type climate of southern Australia. Its deep-rootedness and drought tolerance offer many benefits. Animal production benefits include extending the growing season, by providing green feed in late spring and summer and increasing soil fertility, through its ability to fix nitrogen. Environmental benefits include better utilisation of water at depth than annual species, prevention of secondary salinity through a reduction in ground water recharge and mitigation of rising water tables, and a reduction in the risk of soil erosion.

Tedera is viewed as a more drought tolerant alternative to lucerne, for soils in which lucerne does not perform well. It is more tolerant of acid soils than lucerne and there are some indications of it having higher tolerance of salinity and waterlogging (D. Real, personal communication). However, more research is needed to determine the range of soil types suited to tedera. Extensive grazing studies are also required to determine the best management strategies.

1.2 Germination and emergence factors

In order to introduce tedera into the farming systems of southern Australia, an understanding of its seed biology and germination requirements is needed to determine optimum sowing conditions and to identify any potential constraints to its establishment. The potential use of seed priming with plant signalling compounds also requires investigation, to determine whether they can increase germination and emergence success of tedera.

Fundamental information is needed on water uptake (imbibition) and dormancy characteristics and the temperature, light and moisture requirements for germination. This information is required to determine the best time to sow in different regions. Information is also needed on the depths that seeds will emerge from to enable recommendations for sowing depth to be made. In southern Australia, lucerne is often sown from late autumn to late winter, to enable good establishment and root growth before the onset of the summer drought period (Moore, Sanford, and Wiley 2006). It is expected that a similar sowing time will be appropriate for tedera. Tedera seed is quite large compared to lucerne, suggesting it may be easier to establish. Lucerne is best sown at a depth of 5-10 mm, but the larger seed size of tedera suggests it could be sown deeper.

One factor that may be a limitation to the ease of sowing tedera, is the beak structure on its seed, and its hairy fruit. It is expected that these two characteristics will reduce flowability of the seed through conventional seeding equipment.

The beak can be readily removed by mechanical harvesting and thrashing of the seed, leaving only the oval shaped seed, but the effect of this removal on germination needs to be investigated. Most legumes have an impermeable seed coat (hard seeds) as a physical dormancy factor, which prevents out of season germination and provides seeds for germination in subsequent seasons. There is no information on the level of hardseededness in tedera. This information is crucial, in order to determine the need for mechanical scarification of the seed coat to improve germinability prior to sowing.

This research was conducted to investigate factors influencing germination and establishment of tedera. It was conducted by Mr Cale Beard as an Honours project through Curtin University of Technology, as part of the Future Farm Industries CRC project “Reliable establishment of non-traditional perennial pasture species”, with additional funding from Meat & Livestock Australia, Australian Wool Innovation Ltd and the former Land and Water Australia. The project was co-supervised by the Department of Agriculture and Food Western Australia (DAFWA) and Kings Park Laboratories.

2. 2. Aims and objectives

The aim of this project was to study the germination characteristics of tedera to gain an understanding of its seed biology and germination requirements, in order to determine optimum sowing conditions and identify any potential constraints to its establishment.

Key objectives were to:

- Assess initial seed quality and seed imbibition characteristics to determine if dormancy or other restrictions to germination were present;
- Determine the effect of seed manipulation techniques on germination;
- Identify optimum temperatures for germination;
- Determine the effect of light on germination;
- Examine whether priming seeds with plant signalling compounds (PSC) enhanced germination;
- Investigate the effects of moisture and saline stress on germination; and
- Determine optimum sowing depth

Two accessions (numbered 2 and 6) under evaluation in the Future Farm Industries CRC tedera breeding program, were used. Accession 2 originates from Famara, on the island of Lanzarote (150-300mm annual rainfall), while accession 6 is from Teno, on the island of Tenerife (300 mm annual rainfall) (D. Real, pers. communication).

3. Seed quality and imbibition

3.1 Introduction

Seed quality characteristics, such as seed size and seed viability, will have a major influence on germination and establishment. Seed quality is important to understand as it reflects the germinability of the seed and its correlation with germination percentages. Seed size is important, as it is correlated with the seed's ability to emerge from depth and the amount of seed storage reserves for seedling growth (Powell 1988). Also affecting seed quality at germination is any period of after-ripening dormancy the seed may possess. After-ripening periods are common in many plant species and regulate the timing of germination. Two key factors that influence after-ripening period of the seed are temperature and seed moisture content (Fenner 2000). Dry storage can increase the rate of physiological changes and can result in a decline in the level of innate dormancy, or time of after-ripening (Fenner 2000), and alternate storage conditions can be integrated, such as changes in relative humidity and temperatures, if required to minimise this period.

Seed imbibition to critical seed water content (SWC %) is necessary for a seed to germinate. There are three phases of water uptake, initial uptake, lag phase and growth phase, and the transitional period from phase 2 (lag phase), to 3 (growth phase) is the critical level of water content that the seed requires to germinate (Benech-Arnold and Sanchez 2004; Khan 1982).

In this experiment the basic seed characteristics (seed size and seed quality) of both accessions of tedera were studied, to determine any differences in the attributes that may limit their adaptability to the Mediterranean-type environment. Furthermore, after-ripening period and seed imbibition were studied. The imbibition test will also determine if the seed possesses dormancy characteristics, such as physical (restriction to water uptake) or a physiological (permeable to water uptake but with a physiological restriction) dormancy (Baskin and Baskin 1998).

Germination is defined as the initial water uptake of the seed and the physiological processes that occur within the seed in preparation for it to germinate. However, this is impossible to see until there is the protrusion of the radicle through the seed coat; visible germination (Bewley and Black 1994). Germination will not occur until dormancy state has been relieved. Once dormancy has been overcome, seeds are ready to germinate, subject to optimal temperature, moisture and light conditions (Baskin and Baskin 1998). These are the three most important factors, although there are further methods of increasing germination, such as plant signalling compounds (PSC). The influence of these factors can be readily examined in the laboratory. For the simplicity of this thesis, germination is defined as the protrusion of the radicle through the seed coat.

3.2 Materials and Methods

3.2.1 Seed material

Tedera seeds from two accessions; accession 2 originally from Lanzarote, and accession 6 originally from Tenerife, were collected by hand on the 15th December 2008 from a trial at the Department of Agriculture and Food Western Australia (DAFWA) Medina research station. Seeds were stored in a 25% relative humidity controlled drying store at 16°C until used. Experiments commenced in February, two months after collection, for most of the experiments, however the after-ripening experiment began at harvest.

3.2.2 Experimental design

Seed quality

Both accessions of tedera were compared for size differences using four replicates of 50 seeds. Seeds were weighed in grams to three decimal places, and a mean weight derived. The accession with heavier seed (accession 2) was used for the imbibition experiment, as it is reported the heavier seeds would perform better, with increased seedling vigour (Forbes and Watson 1992). Before each experiment all seeds were x-rayed (low energy X-radiography machine) to ensure 100% seed fill. Dry weights were recorded after seed removal from the dry store.

After ripening

Only accession 2 was used in this experiment. Four replicates of 15 seeds were placed into petri dishes and incubated at a constant 20°C, 12 hourly light/dark. Seeds were germinated on filter paper with tissue culture (TC) grade filtered water, ensuring enough moisture to eliminate water as a constraint. The petri dishes were then wrapped in parafilm to prevent evaporation. Seeds were left to imbibe for 28 days and germination was recorded twice weekly. After 28 days, germination percentage was calculated for each experiment. Seeds that did not germinate were exposed to tetrazolium chloride (TZ) to determine if seeds were still viable (Appendix 10.2). The after-ripening experiment was repeated at three different time periods; 15th December, 5th January and 3rd March. In order to prevent fungal growth, seeds were bleached prior to each experiment using calcium hyperchloride 4% (Appendix 10.1).

Seed imbibition

Imbibition was measured in accession 2 after initial seed weights were calculated. Five seeds were used per replicate, with four replicates for each treatment. As requirements for seed imbibition can be dependent on the size of the seed, along with other physical properties such as protein or fat content (Benech-Arnold and Sanchez 2004), the seed weights of each replicate were selected to be greater than 30 mg to ensure the accuracy of the three-point scales. Three imbibition treatments were used; awn, deawned, and awn + cut. Tedera seed possess awns that can easily be removed with tweezers. The cut treatment involved using a surgical scalpel to protrude the seed fruit and coat of an intact seed, without removing the coat and without damaging the embryo. An initial seed weight was recorded for each replicate. Seeds were imbibed on filter paper in petri dishes with TC grade water, at room temperature (approximately 20°C). Successive measurements were taken after 30 minutes, 1, 2, 4, 24, 48 and 72 hours. At each extraction seeds were dried on paper towels and left to air dry for 2-3 minutes before being re-weighed. Fresh weights were recorded for each replicate. Seeds were then returned to the imbibition environment until the next extraction. After 72 hours, seeds were placed into foil containers and into a drying oven set at 107°C for 17 hours to determine seed moisture content using International Seed Testing Association (ISTA) standards (*International rules for seed testing* 2007). The seeds were then re-weighed for final dry weights. Percent seed water contents from fresh weights and final dry weights were then calculated.

3.2.3 Assessment of germination

Seeds were classified as germinated if there was a healthy radicle protruding (>3 mm). Seeds that had germinated were left in the petri dish to ensure that they would develop a healthy coleoptile.

3.2.4 Data analysis

Germination percentage for each replicate was calculated as the percentage of germinated to total seeds. Mean and standard error were calculated as follows.

Mean = Σ percentage germination / *n*

SE = SD/√*n*

Where; SE = Standard error; SD = Standard deviation; *n* = number of replicates

Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

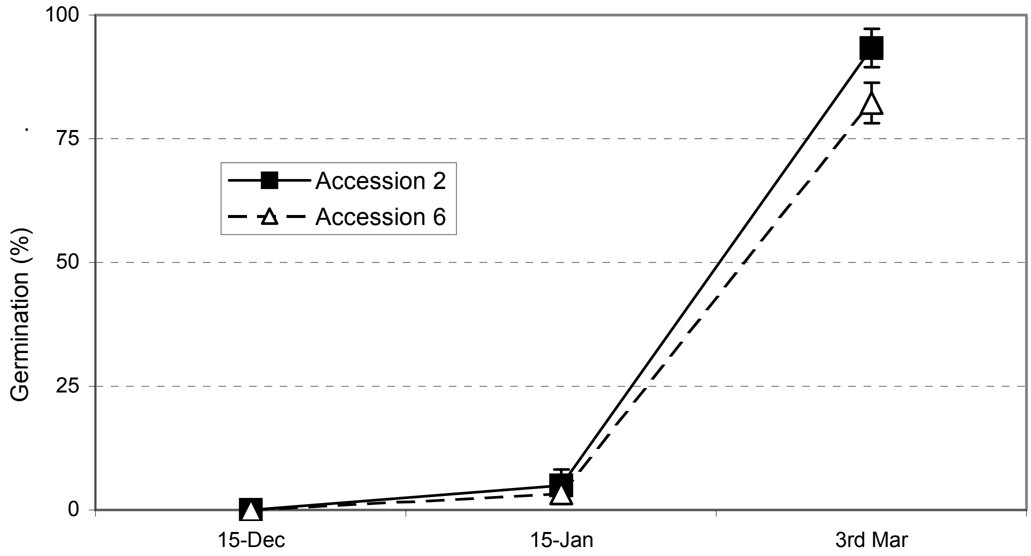
3.3 Results

Seed quality and after-ripening

The two accessions produced different seed weights. The 50 seed sample of accession 2, weighed 1.18g, compared to 1.01 for accession 6.

Tedera seeds required a period of around three months to produce high germination percentages (Fig. 2.1). No germination occurred at for both accessions, and after one month germination increased only to 5 and 3%, accession 2 and 6 respectively. After three months accession 2 and 6 produced germination means of 93 and 82% respectively. All seeds that did not germinate in the period of after-ripening were still viable after TZ tests.

Figure 2.1. After-ripening period of two accessions of tedera.



Seed imbibition

Tedera seeds imbibed water to a critical seed water content percentage (% SWC) of 48-50%, which resulted in germination (Fig. 2.2). The with awn treatment did not have any germination after 72 hours, although the critical SWC% had only just been reached. The awn + cut treatment produced the fastest imbibition to the growth phase, in 24 hours. The awn and de-awned treatments did not differ significantly, and reached the critical SWC % by 72 hours. The imbibition experiment showed that tedera seeds did not possess a physical dormancy characteristic, as they were permeable to water.

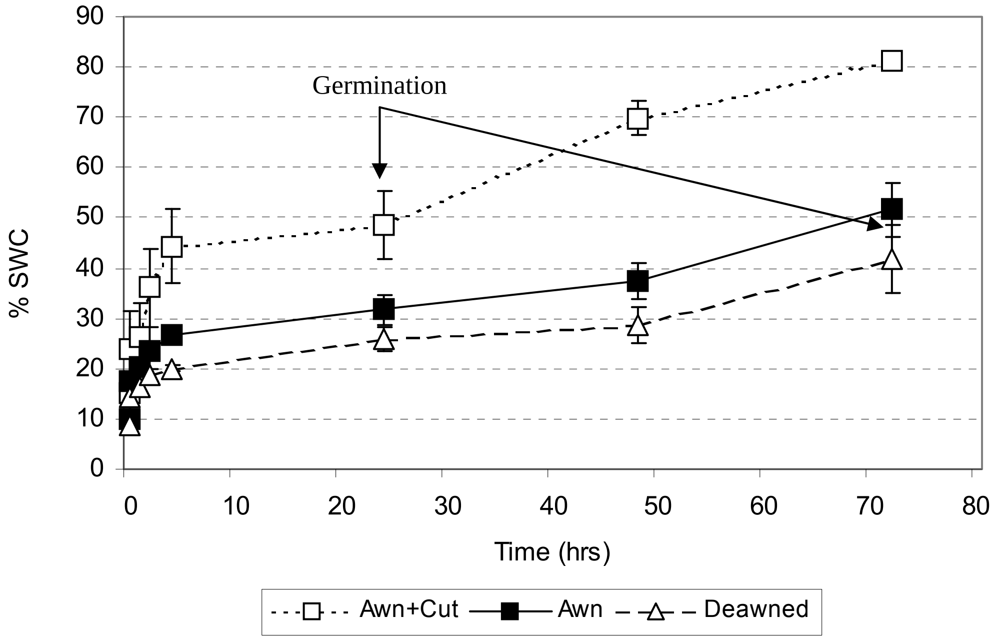


Figure 2.2: Seed imbibition of tedera seeds over time. Treatments used were awn + cut, plus awn, and minus awn. Bars indicate 1 s.e. of the mean.

3.4 Discussion

The experiments indicate that tedera requires a critical seed water content of 48-50% to germinate. This suggests that tedera is best sown in the winter months of the Mediterranean-type environment when water is non-limiting. The germination rate is an important aspect of tedera as it will determine the time period in which adequate soil moisture content is required for tedera to germinate. When the seed was cut, critical SWC% leading to germination occurred after just 24 hours. However, even though seeds that had no coat manipulation took longer to germinate, they were able to germinate after 72 hours, indicating that tedera seeds do not have physical dormancy. It is likely that tedera has a non-deep physiological dormancy classification, as there appears to be a restriction of the radicle to protrude the seed coat. As seen with the results, manipulation of the seed coat, such as the cut, can relieve the restriction of the seed coat for the radicle to develop.

An after-ripening period, or period of primary dormancy, appears important for regulating the germination of tedera. The period from seed maturation to the time when there is a significant germination percentage for effective establishment can have huge impacts on the commercial aspects of the seed, such as time to sale or use. The experiment showed a three-month period where germination was restricted by primary dormancy and required a period of after-ripening. Some seeds are able to produce high germination percentages immediately after maturation, and in other cases some seeds will produce very low germination percentages immediately after maturation (Baskin and Baskin 1998). Similarly, some seeds that are exposed to dry conditions after maturation, i.e. dry storage, can decrease germinability substantially (Baskin and Baskin 1998).

Seed size can have subsequent benefits for germination, such as improved chances of emergence from depth and increased seed storage reserves for greater seedling vigour (Powell 1988). The difference in seed weights was not large between the two accessions, so differences in the performance of the seeds would be expected to be minimal.

The seed characteristics studied in this experiment give a good indication of the potential germination of tedera. There are numerous other factors that can limit the amount of water available for the seed to uptake and thus germination rate, such as soil type and soil characteristics, however, this experiment gives an insight into the base SWC% required to successfully germinate. Seed manipulation obviously has an important impact on the rate of water uptake and germination, however, a treatment such as the cut, is not necessarily practical for a large-scale operation. Consequently, experiment two focuses on seed manipulation techniques that could be used on a larger scale to try and improve water uptake and germinability.

4. Germination response to seed manipulation, threshing and scarification

4.1 Introduction

Seed manipulation in this experiment is referred to as the abrasion of the seed coat by nicking (cut) with a surgical scalpel. Scarification of the seed coat is widely used to release physical dormancy, such as hardseededness, in many legume seeds, but it can also increase germination percentage and rate in seeds with physiological dormancy (Baskin and Baskin 1998; Forbes and Watson 1992). Chapter 2 showed that tedera seeds do not have physical dormancy as they readily imbibe water, but there are significant differences in germination rates between the treatments used. In the previous chapter, it was observed that the awn + cut treatment increased water uptake and time to germination, which may have agronomic and environmental benefits, such as more efficient use of available water and improvements in seed vigour to establishment, although most of which depends on the seed and the species adaptive strategy. For example, Turner et al. (2005) suggested that the environment in which the species is native regulates the water uptake; so a lower water uptake is seen in species from dry or arid areas. Such a treatment (cut) is not practical for treating a large number of seeds, and also involves a high risk of seed damage. Threshing and scarifying are two less invasive methods of manipulating the seed coat as a means of increasing water permeability and assisting radicle protrusion. Threshing is a typical outcome from a conventional harvesting operation in broadacre farming, through the removal of seed from plant material, however scarification can be implemented before commercial release of seed. It is evident that cutting the seed can increase the germination rate, however the germination percentage benefit is unclear, and this experiment will determine the best method of manipulating the seed to improve germination percentage. This experiment explores the effect of seed manipulation, either via seed cut, threshing or scarification on germination.

4.2 Materials and methods

4.2.1 Seeds

Seeds of tedera accessions 2 and 6 were hand harvested from the DAFWA research station in Medina (see Section 2.2.1 for more details).

4.2.2 Experimental design

Seeds from both accessions were counted out into 8 lots of 60 seeds to produce 8 treatments x 3 replicates x 20 seeds per replicate. Seeds were either left intact or mechanically threshed prior to any further manipulation. Seeds were threshed using a Venables threshing machine (Kimberly Seeds, Perth). Seeds were cut using a surgical scalpel, only slicing the outer seed coat. Scarified seeds were produced by five passes of rubbing with coarse sandpaper on a rubber mat. This resulted in predominantly only one side of the seed being scarified.

The treatments for both accessions are shown below in the table; where N = raw seed, scar = scarified, and T = threshed.

Table 3.1. Seed manipulation treatments.

No.	Description	Treatment label
1	Unthreshed seed with awn	N + awn

2	Unthreshed seed minus awn	N + no awn
3	Unthreshed, scarified seed minus awn	N + scar + no awn
4	Unthreshed, cut seed minus awn	N + cut + no awn
5	Threshed seed with awn	T + awn
6	Threshed seed minus awn	T + no awn
7	Threshed, scarified seed minus awn	T + scar + no awn
8	Threshed, cut seed minus awn	T + cut + no awn

On the 4th of August seeds were placed on filter paper in petri dishes with 9mL TC grade water, and incubated at 20°C, 12 hour light/dark. All seeds were x-rayed prior to plating to ensure full seed, and were also bleached using 4% sodium hyperchloride for 15 minutes prior to manipulation to prevent fungal growth (Appendix 10.1). Petri dishes were sealed with parafilm to prevent evaporation. The seeds were left in the temperature-controlled incubators for 14 days and germination scored every few days.

4.2.3 Assessment of germination

Seeds were classified as germinated if there was a healthy radicle protruding (>3mm). Seeds that had germinated were left in the petri dishes to ensure they would form a coleoptile. Germination was scored regularly until day 14, when final germination percentage and germination rate were calculated.

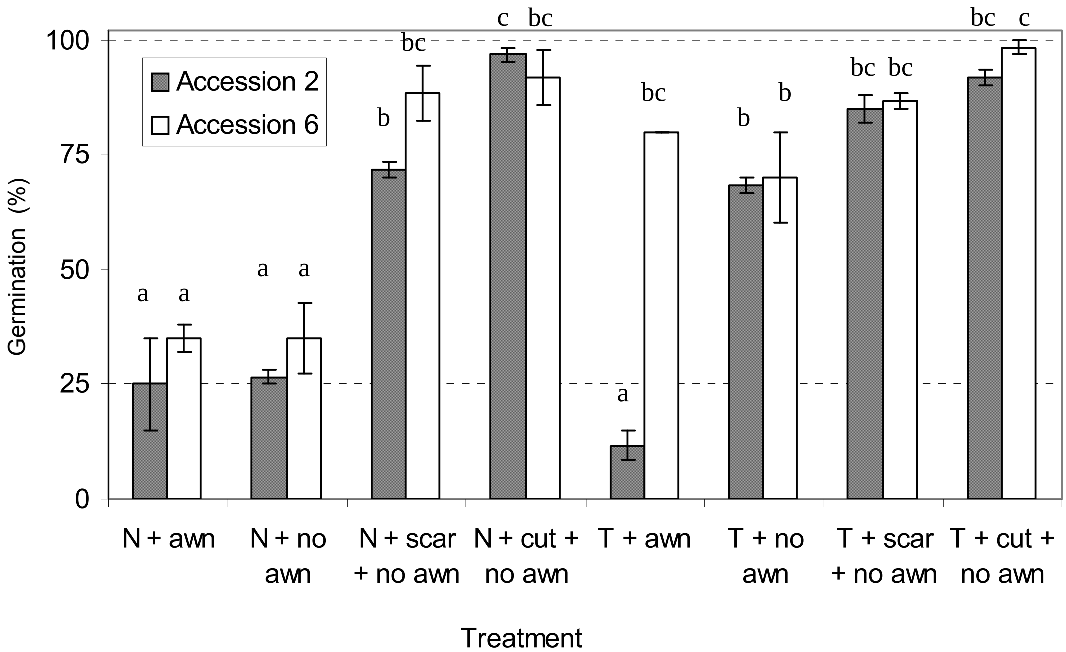
4.2.4 Data analysis

Germination data for each replicate was calculated as a percentage of germinated to total seeds (20). Mean and standard error for each of the treatments were calculated as in Section 2.2.4. Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

4.3 Results

Germination percentage

For both accessions 2 and 6, seeds that were cut and de-awned (cut + no awn) produced the highest germination (mean >90%) regardless of whether they were threshed or not (Fig. 3.1). Threshed and scarified seed had the second highest germination (>75%) and other treatments slowly declined. The accession 6, threshed + awn treatment produced the lowest germination percentage (12%). Normal seed, ± awn, had the next lowest germination means. A significant difference occurred between accession 2 and 6 only in the threshed + awn treatment. This reason for this difference is unknown. Manipulation treatment used had a significant affect on germination of tedera ($p<0.001$), but accession did not, apart from the threshed + awn treatment. The only significant differences between accessions ($p<0.05$) was the treatment: threshed + awn, with >68% difference in mean. Within accessions there were significant differences between most treatments i.e. normal seed + scar + no awn was significantly higher than normal seed ± awn. The awn had no significant effect on germination percentage.



Seed manipulation had a significant impact on the germination rate of tedera (p<0.001) (Fig. 3.2). Accession 6, threshed + cut + no awn produced the highest rate of germination at 33% germination per day, followed by accession 2 with the normal + cut + no awn treatment. The cut treatments produced the highest rates of germination, followed by the scarified (either normal or threshed seed) treatments. Normal seeds with ± awn produced the lowest rate of germination, and similarly accession 2 threshed +awn, and all factors; ± awn, cut, and scarified had a significant impact on germination rate (p<0.001). Similarly accession had a significant impact (p=0.041), although to a lesser extent. The only significant difference between accessions was the threshed + awn treatment. All other treatments were not significantly different between accessions. Table 3.2 shows the means differences using Tukey's (HSD) between treatments and accessions. Treatments joined by a common letter are not significantly different.

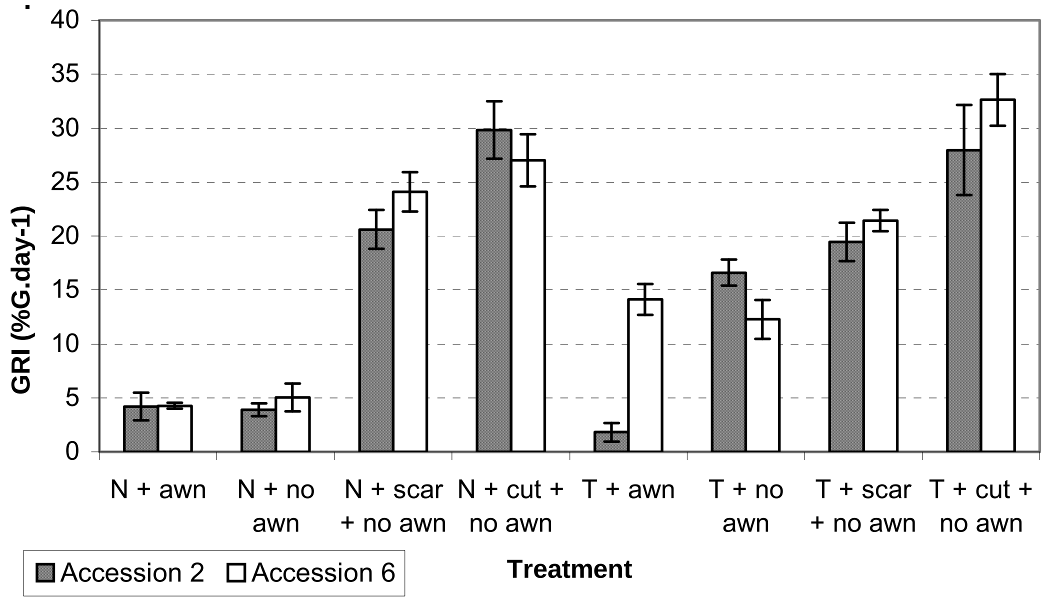


Figure 3.2. Effect of seed manipulation, threshing and scarification treatments on the growth rate index (GRI) of two accessions of tedera after 14 days. Bars indicate 1 s.e. of the mean.

Table 3.2. Mean differences between seed manipulation treatments on germination rate of tedera.

Seed manipulation			
Treatment	**Accession**	**GRI mean**	**HSD**
N + awn	2	4.18	abc
N + awn	6	4.25	abc
N + no awn	2	3.90	ab
N + no awn	6	5.03	abc
N + scar + no awn	2	20.61	defg
N + scar + no awn	6	24.09	efgh
N + cut + no awn	2	29.83	gh
N + cut + no awn	6	26.99	fgh
T + awn	2	1.81	a

T + awn	6	14.11	cde
T + no awn	2	16.62	de
T + no awn	6	12.28	bcd
T + scar + no awn	2	19.46	def
T + scar + no awn	6	21.41	defg
T + cut + no awn	2	27.97	fgh
T + cut + no awn	6	32.62	h

4.4 Discussion

The previous chapter identified that seed manipulation had a positive effect on the germination rate of tedera. The results of this experiment confirmed this finding. Seed manipulation in many forms (i.e. cutting, scarifying, threshing) improved germination percentages and rates, however, the most important treatment was the threshed seed + cut + no awn, which produced the highest germination. Within the normal seed experiment, the cut + no awn treatment had a significant increase in germination percentages compared to the untouched seed. De-awning the seed did not appear to have an effect on germinability, therefore indicating the important treatments are scarification or cutting. However, as mentioned in the previous chapter, the cut treatment is not necessarily practical prior to commercial release, due to the risk of damaging the seed and labour intensity. Scarifying seeds will be much more practical.

In making recommendations for the optimal method of seed manipulation to increase the potential of tedera to germinate, and in a shorter period of time, a cut treatment would obviously be the best, however it was impractical. Scarification of the seed using sandpaper is a more practical and simpler method of seed manipulation that produced high levels of germination in both accessions (>70%). Scarification can easily be implemented in a large-scale operation prior to commercial release, and the risk of seed damage is less than that of cutting. This process involves scarification through coarse surfaces, such as sandpaper, to scratch the surface of the seed to give it weak areas for increased water penetration and aid in radicle protrusion (Baskin and Baskin 1998). Threshing did also have a noticeable benefit on mean germination percentages and rates, especially in the, + awn, and, + no awn treatments. Threshed seed is generally the result of conventional harvesting of seed crops. The seed goes through a process of de-hulling and separating seed from plant material. This process will have a manipulative influence on the seed coat to varying extents, and this seed product could still be used to potentially produce greater than 75% germination percentages, as seen in the results (apart from accession 2 + awn). The difference in germination of the accession 2 threshed + awn treatment is unknown, considering in all other manipulation treatments there was not much difference in accession means. Further research on this aspect is required to determine whether it is a characteristic of accession 2 that it does not respond to the threshed + awn treatment as well as accession 6, or if it was just experimental error. Through the threshing process, greater than 90% of the seed will have the awn removed, so the limitations within accession 2 with the awn remaining will be minimal. These seeds can also be put through a scarifying machine to increase the germinability of the seed. This is an important finding necessary to understand to be able to prepare the seed for commercial release to producers.

5. The effect of temperature and light on germination

5.1 Introduction

Temperature and light are two key factors that can influence both dormancy status and release, and also germination. Temperature is the most important factor that impacts on breaking of dormancy and seed germination (Baskin and Baskin 1998; Cochrane and Probert 2006; Mott 1972), whereas light stimulates germination (Vleeshouwers et al. 1995). Seeds vary in their germination time according to the environmental and climatic conditions present, and to which the plant has adapted. The timing of germination in relation to temperature is also influenced by the dormancy characteristics of the seed. Some seeds require specific temperatures (both high and low), and temperature fluctuations to release dormancy (Baskin 2003).

Mediterranean pasture species have been shown to germinate across a wide range of temperatures, and more specifically annual clovers will have optimum germination at 15°C (Norman et al. 1998). Subterranean clover is an annual legume of WA, and similar seed softening are required to that of perennial legumes. Increasing the diurnal temperature (difference between maximum and minimum daily temperatures) exposure, from the average Mediterranean WA range of 15°C, to a range of up to 40°C (as seen in some areas of WA) will significantly increase the rate of softening of the seed (Taylor 2005), thus increasing imbibition. Hill and Luck (1991) also showed that temperature changes had no significant effects on germination percentage or rate in the perennial legume, lucerne. This study examines a wide range of temperatures from 5 to 35°C were used to give a broad scope of the impacts on germination, as well as three alternating temperatures, 20/10°C, 26/13°C, and 35/20°C to reflect natural day/night temperature fluctuations.

Light is one characteristic that is known to have variable impacts on seed germination. Some seeds can germinate without the presence of light, whereas others require a little too significant exposure for good germination. Light is a regulator of germination and not definitively associated with regulating dormancy, unlike temperature (Vleeshouwers, Bouwmeester, and Karssen 1995), however light is known to stimulate germination and aids in terminating dormancy in most species, all of which depends on the definitions of and clarifications of the processes of dormancy and germination (Finch-Savage and Leubner-Metzger 2006). Seeds that are deposited in the soil, and have dormancy characteristics, will either inherit primary or secondary dormancy, or in further studied cases, dark dormancy. Dark dormancy is known as dormancy induced by the lack of light (Pons 1991). The seeds that require light for germination, even if dark dormancy has been induced, can still germinate in the soil if exposed to doses of light at different stages i.e. during cultivation. The absence of light can be fatal in some cases if seeds are buried and do not have access to light, reducing the soil seed bank. Similarly, seeds that have been exposed to levels of red or far-red proportion of light may still germinate in darkness (Pons 1991). In native Western Australian species there is significant increases in germination under dark conditions, possibly in relation to the need to be buried in the soil to increase seed longevity and build seed banks. Even though this is the case, there are further environmental influences that will impact on germination, such as temperature and moisture change and availability. In the same native species, the light inhibition of the seed was more apparent with high temperatures (Bell et al. 1995).

From the literature, it is expected that tedera will be able to germinate at a range of temperatures, and will not be affected by the absence of light, as Hill and Luck (1991), and Baskin and Baskin (1998), have both recorded nil effects from these factors in the germination of nondeep physiological dormancy species and from lucerne. This experiment will provide good information on the ability of tedera to germinate at a range of temperatures, therefore its suitability to specific environments, and coinciding with the imbibition experiment strong recommendations for time of sowing can be made.

This experiment investigates the impact of temperatures at constant and alternating 12-hour light/dark, and full dark treatments on the germination of tedera. In chapter 3, the de-awned + cut treatment had the best germination percentages in both accessions; so to increase the germination potential of the seeds, the same manipulation method was used. Two total dark treatments, 15 and 20°C, were also used to compare from the 12-hour photoperiod treatments. Literature shows that nondormant seeds will germinate in a wide range of temperatures (Baskin and Baskin 1998), and from previous experiments, tedera has no significant dormancy characteristics restricting germination, although nondeep physiological dormancy characteristics are existent, which can be overcome by seed manipulation, which is why the seeds have been surgically cut (nicked). Nondormant seeds are able to germinate equally well in light or dark conditions (Baskin and Baskin 1998). *Trifolium stoloniferum* (perennial) and *Trifolium campestre* (annual) both in the *Fabaceae* family, had no effect of light or darkness on germiantion (Baskin and Baskin 1988). Darkness was used in this experiment to determine if tedera has different germination requirements to other species.

5.2 Materials and Methods

5.2.1 Seed collection

Seeds of tedera accessions 2 and 6 were hand harvested from the DAFWA research station in Medina (see Section 2.2.1 for more details).

5.2.2 Experimental design

Both accessions 2 and 6 were used in this experiment. Seeds were counted out into 36 lots of 20 seeds, so that there were 12 temperature regimes with 3 replicates and 20 seeds per replicate. Temperature treatments consisted of seven constant temperatures (5, 10, 15, 20, 25, 30 and 35°C), and three alternating day/night temperatures (20/10, 26/13, and 35/20°C). All temperature treatments were conducted under alternating 12 hourly light/dark periods, except for the inclusion of two total dark treatments at 15 and 20°C. All seeds were germinated on filter paper in petri dishes with 9 mL TC grade water. Seeds were de-awned and cut, to maximise their potential for germination. Seeds were x-rayed for 100% seed fill prior to plating. The seeds were also bleached using 4% sodium hyperchloride for 15 minutes prior to cutting to reduce the risk of fungal attack (Appendix 10.1). Petri dishes were wrapped in parafilm to prevent evaporation, and dark treatments were covered in aluminium foil to omit light. All petri dishes were placed in temperature-controlled incubators for 14 days. Seeds that did not germinate were exposed to tetrazolium chloride to determine if they were still viable (see Appendix 10.2). Accession 2 was incubated from the 26th of June, and accession 6 was incubated from the 3rd July.

5.2.3 Assessment of germination

Seeds were classified as having germinated if they had a healthy radicle protruding (>3 mm). Seeds that had germinated were left in the petri dishes to ensure they would form a coleoptile. Germination was scored regularly (3 times weekly) until day 14, with dark treatments being checked in dark conditions to avoid the exposure to light. Final germination percentage and germination rate were calculated at day 14.

5.2.4 Data analysis

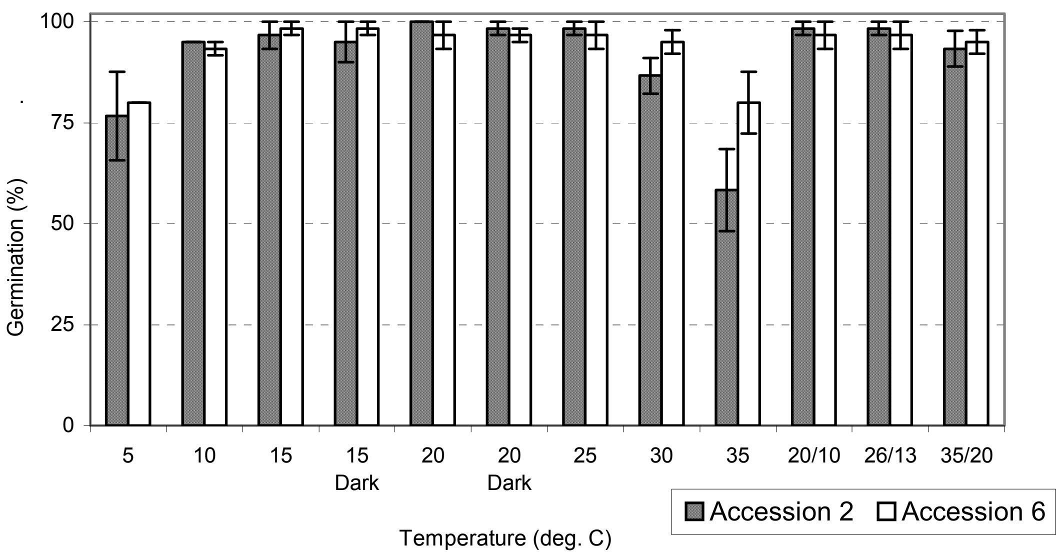
Germination data for each replicate was calculated as a percentage of germinated to total seeds (20). Mean and standard error for each of the treatments were calculated as in Section 2.2.4. Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

5.3 Results

Germination percentage

Figure 4.1 shows the effect of different temperature and light regimes on the germination percentage of the two accessions after 14 days. The seeds incubated between 15 and 25°C, and alternating temperatures at 20/10°C and 26/13°C, produced high total germination means (>95%). Accession 2 at 10°C also produced >95% mean germination. Seeds incubated at temperatures of 10 and 30°C also produced >90% germination in both accessions. Germination percentages declined as the temperatures decreased below 15°C, and similarly as the temperatures increased above 25°C germination percentages declined. At 5°C, germination percentages were still above 75%, whereas temperatures >30°C caused a decline to below 60%. Alternating 12-hourly temperatures did not affect germination percentages with all producing >90% germination. The absence of light at 15 and 20°C also did not affect germination percentages, with both light and dark treatments recording similar mean germination percentages. There were no significant differences between accessions ($p>0.05$), however temperature did have a significant impact on germination differences ($p<0.001$).

Figure 4.1. Effect of different temperature and light regimes on the germination percentage of two accessions of tedera after 14 days. Germination means are significantly different if not joined by a common letter. Bars



indicate 1 s.e. of the mean.

Germination rate

Accession 6 produced the highest germination rate of 31% germination per day, at a constant temperature of 20°C (both light treatments) and at 26/13°C (Fig. 4.2). Accession 2 was not significantly different to accession 6 in these treatments and produced similarly high rates. 5°C produced the lowest germination rate in both accessions 2 and 6, at 6 and 8% respectively. The absence of light had no significant impact within or between accessions. Similarly, alternating temperatures had no significant impact on germination rate, either within or between accessions and produced high germination rates, similar to the cumulative degrees (15, 20 and 25°C). Accession and temperature, had a significant impact on germination rate ($p<0.001$). Accession 6 had a higher GRI overall, although there were no significant differences between accessions at any one temperature. Table 4.1 shows the Tukeys (HSD) between treatments and accessions.

Figure 4.2. Effect of different temperature and light regimes on the growth rate index (GRI) of two accessions of tedera after 14 days. Bars indicate 1 s.e. of the mean.

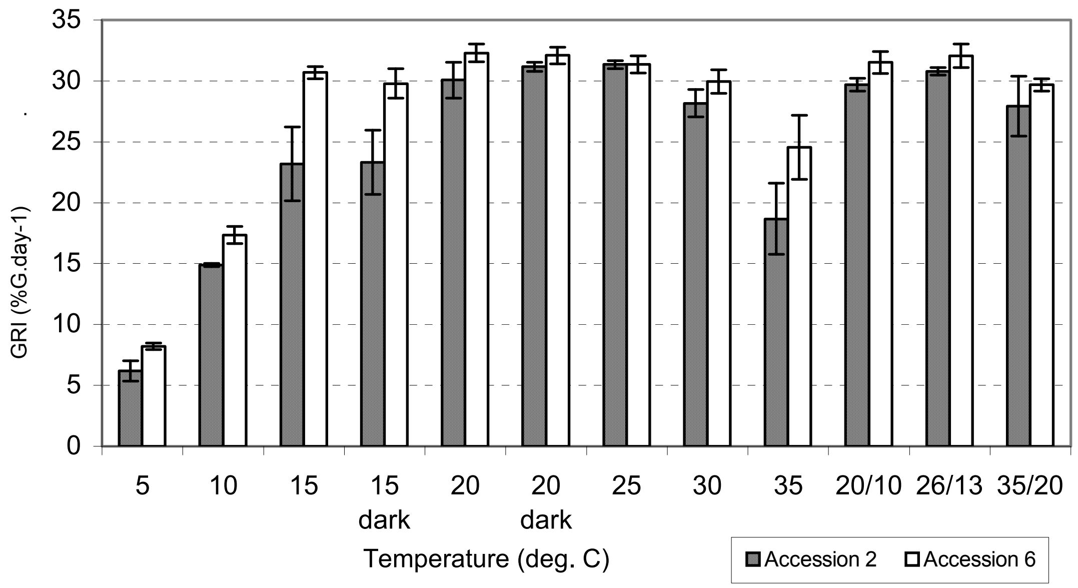


Table 4.1. Tukeys HSD of temperature and light on germination rate of tedera.

Temperature			
Treatment (°C)	**Accession**	**GRI mean**	**HSD**
5	2	6.21	a

5	6	8.21	ab
10	2	14.88	bc
10	6	17.33	cd
15	2	23.19	de
15	6	30.68	efg
15 dark	2	23.32	def
15 dark	6	29.79	efg
20	2	30.06	efg
20	6	32.29	g
20 dark	2	31.16	fg
20 dark	6	32.10	g
25	2	31.35	g
25	6	31.37	g
30	2	28.17	efg
30	6	29.96	efg
35	2	18.68	cd
35	6	24.54	defg
20/10	2	29.69	efg
20/10	6	31.53	g
26/13	2	30.78	efg
26/13	6	32.06	g
35/20	2	27.93	efg
35/20	6	29.67	efg

5.4 Discussion

All temperatures, apart from accession 2 at 35°C, produced over 75% germination, with seeds germinated at temperature ranges 15-25°C producing over 95% germination. Germination at the extreme ends of the range did not have much of a difference in final percentage means, although standard errors at 5°C (excluding accession 6) and 35°C temperatures were greater than those at any other constant temperature. These standard errors ranged from 7% in accession 6, 35°C, and up to 11% in accession 2, 5°C. This demonstrates there could be large variability in germination within a broadacre program if temperatures were at these extremes, however, it is unusual in the Mediterranean-type environment for soil to maintain these temperature extremes for long periods of time, especially during the winter months.

Whilst constant temperatures did not show many differences in final germination percentages, there were significant differences in the germination rate. At 5°C, germination rates were only 6 and 8% germination per day for accession 2 and 6 respectively, significantly lower than those at temperature ranges of 15 to 30°C, which were between 23 and 33%. This is an important finding as in the WA environment it is important for seeds to germinate quickly to take advantage of available soil moisture, as long as dormancy traits are overcome (Mott 1972), and seeds at 5, 10, and 35°C were lower than between 15 to 30°C. It was expected that there would be higher germination percentages and rates between the 15 and 20°C, after studies from Norman et al. (1998) showed that annual clovers had highest germination at 15°C, however tedera did expand on that proving that it can also have similar results at warmer 25 and 30°C.

Alternating temperatures did not have any impact on final germination percentage or rate at any range, and results were similar to those at the equal cumulative degrees, 15, 20 and 25°C. Similarly the absence of light had no significant effect on the germination percentage or rate, in the 15 and 20°C treatments that both compared. This was also expected supporting findings in the literature by Baskin and Baskin (1998) reported that light does not have a great influence on germination.

Tedera can be widely adapted to a range of environments where temperatures can range from 5 to 35°C and still achieve germination percentages above 75%, with optimum germination between 15-25°C. Germination rates however can be more of a restriction in the WA environment due to the requirement of at least 48-50% SWC required for germination, and at 5 and 10°C, the period for this amount of moisture being available for the seed to be able to germinate is more extensive compared to the other temperatures. It is also important is to note that seed burial and the absence of light will not prevent germination, indicating that the seed can be drilled into the soil with no adverse effects on seed germination and establishment. This hypothesis will be tested in experiment 6 (Chapter 7).

6. The effect of plant signalling compounds on the germination of tedera

6.1 Introduction

PSC's are known to stimulate germination, and may also overcome regulatory cues such as temperature and light (Bell et al. 1995), and also early vigour. The effectiveness of chemical compounds varies greatly within seed species, however, in most cases the seeds imbibed in the presence of PSCs improve germination rates and percentages (Finkelstein et al. 2008). In herbaceous and Mediterranean plants, including *Apium prostratum, Goodenia stenophylla, Marianthus granulatus, Myriocephalus suffruticosus, and Velleia foliosa,* all GA3 (Cochrane and Probert 2006), smoke water (Tieu et al. 1999) and KNO3 (Cochrane and Probert 2006) have all increased germination.

As seen in the previous experiments, and in particular the temperature and light experiment, germination of tedera is successful over a range of temperature (5-35°C) and light or dark conditions. However, increases in germination rate and subsequently early vigour will be beneficial, especially in the WA environment where conditions for effective germination can fluctuate greatly. In this experiment, the effect of osmo-priming with several plant signalling compounds (PSC) on tedera seed to overcome non-deep physiological dormancy and therefore its impact on germination was studied. PSC's included, H20, smoke water, karrikinolide (KNO3), gibberellic acid (GA3), potassium nitrate (KNO3), and ethylene.

6.2 Materials and Methods

6.2.1 Seed collection

Seeds of tedera accessions 2 and 6 were hand harvested from the DAFWA research station in Medina (see Section 2.2.1 for more details).

6.2.2 Experimental design

Seeds were counted out into eight lots of 60 for accession 2 and seven lots of 60 for accession 6. PSC treatments for both accessions included dry seed (control), H_2O (TC grade), GA_3 (0.28 mM), KAR_1 (the active chemical in smoke) (0.67 µM), KNO_3 (0.30 M), smoke water (1%), and ethylene as ethaphon (20 mM). All seeds were de-awned and cut, however for accession 2 one extra treatment was de-awned with an absence of cutting for priming in KNO_3 to determine if the potassium nitrate has any efficacy in breaking down the seed coat. Replicates were placed into mesh nylon bags, and bulk primed in the different treatments. Priming involved placing seed bags into corresponding PSCs and incubating for 24 hours at 20°C. Once seeds were extracted from the priming solution, they were dried with paper towel, and air-dried for two days. Seeds were then transferred to the seed drying store for four days to return them to their previous dry weights. All seeds were germinated on agar in petri dishes and germinated at 20°C in the 12 hour light/dark controlled incubator. The agar consisted of 0.6 g of agar powder in 100 mL TC grade water. The mixture was made up to 400 mL and autoclaved, and poured once temperature decreased to 60°C. Plant preservative (0.1%) was added to the mixture at 60°C to assist seed survival. This experiment used agar as it was a more sterile test for the chemicals. All petri dishes were wrapped in plastic wrap to prevent the agar drying out, and then covered in aluminium foil to omit light. Seeds were bleached in 4% calcium hyperchloride for 15 minutes to eliminate the risk of fungi development prior to priming (Appendix 10.1). Seeds that did not germinate were exposed to tetrazolium chloride to test viability (Appendix 10.2). Accession 2 was incubated from the 7th of July, and accession 6 from the 13th of July.

6.2.3 Assessment of germination

Germination was considered to have occurred when the seed comprised a healthy radicle (> 3mm). Seeds that germinated were left in the petri dishes to ensure they would develop a coleoptile. Germination was recorded seven times, over a 14 day period, after which germination rates and percentages were calculated.

6.2.4 Data analysis

Germination data for each replicate was calculated as a percentage of germinated to total seeds (20). Mean and standard error for each of the treatments were calculated as in Section 2.2.4. Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

6.3 Results

Germination percentage

The PSC treatments H_2O, smoke water, KAR1 and GA3 all produced similar results to the control treatment with mean germination >90% (Fig. 5.1). Accession 6 performed better, although not significantly different to accession 2 apart from the ethylene treatment, with all of these treatments listed above having ≥95% germination. Imbibing seeds with these compounds had no impact on final germination percentage compared to the control. Germination was low in the KNO3 treatment in both accessions at ≤25%. Overall, treatment had a significant impact on mean germination percentages ($p<0.001$). The ethylene treatment had the only significant difference in accessions ($p<0.05$) of approximately 93%. The KNO3 treatment, without cut, was only applied to accession 2. Germination percentage for this treatment was ≈27%. The cut treatment was not significantly different to the untouched seed.

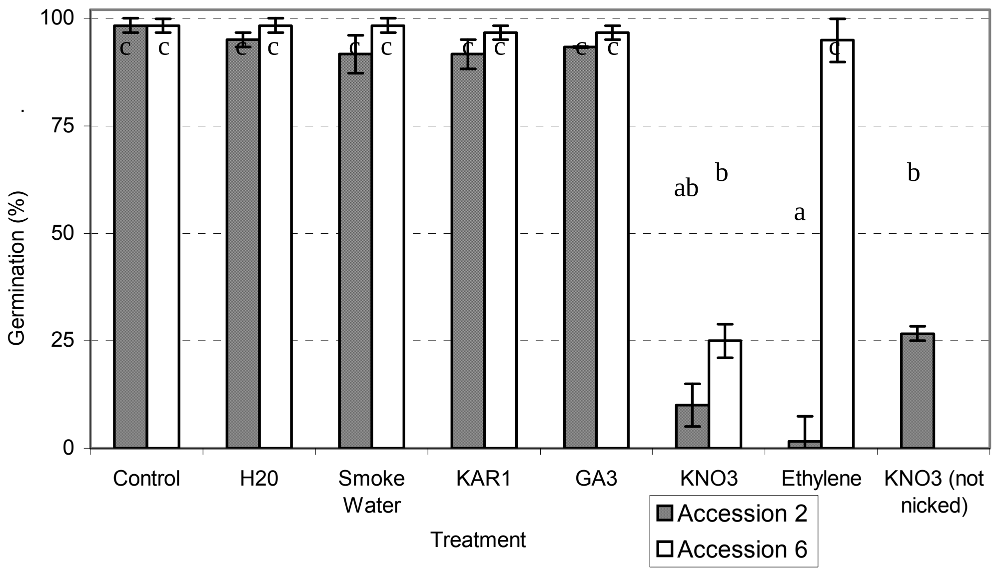


Figure 5.1. Effect of plant signalling compounds (PSC) on the germination percentage of two accessions of tedera after 14 days. Germination means are significantly different if not joined by a common letter. Bars indicate 1 s.e. of the mean.

Germination rate

Accession 2 produced the highest germination rates in 5 of the 8 treatments (Fig. 5.2). The highest germination rate was accession 2 osmo-primed with smoke water (64% germination per day), followed by accession 2 primed with both KAR1 (58%), and H_2O (57%). These were not significantly different. KNO_3, and accession 2 ethylene, produced the lowest germination rates, <7% germination per day. Treatment had a significant impact on germination rate ($p<0.001$), as did accession. There were significant differences between accessions in the treatments, H_2O, smoke water, KAR_1, and ethylene. Smoke water and KAR1 increased germination rate over untreated seed (in accession 2), but no treatments increased rate in accession 6. There is a genotype effect present. There were no significant differences between accessions in the control, GA_3, and KNO_3 treatments. Table 5.1 shows the honest significant differences (HSD) between treatments and accessions.

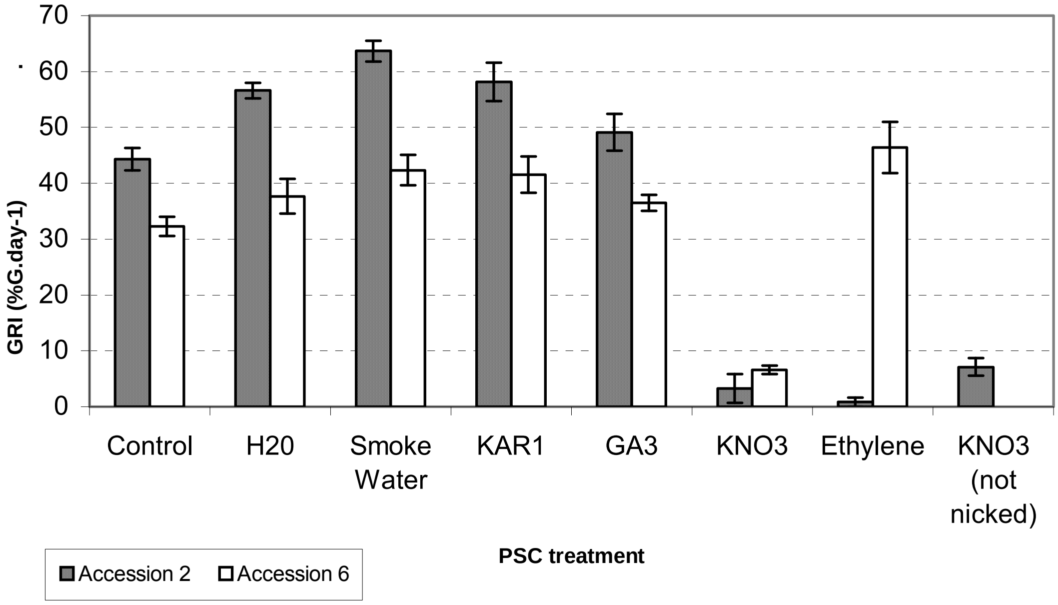


Figure 5.2. Effect of plant signalling compounds (PSC) on the GRI of two accessions of tedera after 14 days. Bars indicate 1 s.e. of the mean.

Table 5.1. Tukeys HSD of plant signalling treatments on germination rate of tedera.

PSC treatment			
Treatment	**Accession**	**GRI mean**	**HSD**
Control	2	44.31	bcd
Control	6	32.27	b
H20	2	56.61	def

H20	6	37.64	bc
Smoke Water	2	63.66	f
Smoke Water	6	42.33	bc
KAR1	2	58.15	ef
KAR1	6	41.54	bc
GA3	2	49.13	cde
GA3	6	36.49	bc
KNO3	2	3.22	a
KNO3	6	6.56	a
KNO3 (not nicked)	2	7.11	a
Ethylene	2	0.83	a
Ethylene	6	46.45	cde

6.4 Discussion

Osmo-priming the seeds in plant signalling compounds (PSC) had a significant ($p<0.001$) effect on germination percentages, this did not differ from the control. Priming for 24 hours in H_20, smoke water, KAR1 and GA_3, and ethylene (accession 6) all produced the same final germination means as the control treatment. This also indicates that tedera will germinate well without the osmo-priming in the PSC, which inturn could mean fewer costs involved with commercial release to growers. On the contrary, germination rates within these treatments did vary significantly ($p<0.001$). The two key PSC treatments were KAR1 and smoke water, which produced greater ($p<0.05$) germination rates compared to the control, increasing rates by 20 and 14% respectively. This is significant in knowledge that areas where rainfall is not as consistent, priming with these chemicals could improve establishment.

The results from this experiment show that different PSC treatments will affect each accession differently. For example, germination percentages are not different, however germination rates are much higher in accession 2 in response to PSC (control, H_20, smoke water, KAR1 and GA_3) treatments compared to accession 6. The reasons behind this could be numerous, and more research could be used to develop accurate reasons about the seed physiology. Interestingly, ethylene produced some very comparative results between accession 2 and 6 (93% difference in final germination and 39% difference in germination rate). It is unknown why this occurred, possibly due to human error with chemical formulation. Potassium nitrate (KNO_3) had a negative effect on germination percentages and rates. This is important as the use of nitrate can promote seed germination by an osmotically induced increase in water uptake (McIntyre 1997), however, it had the total opposite effect in this experiment. Further research needs to explore and expand on this result.

In herbaceous and Mediterranean plants all GA3 (Cochrane and Probert 2006), smoke water (Tieu et al. 1999) and KNO3 (Cochrane and Probert 2006) have all increased germination. Tedera is so versatile that there was no significant correlation, to these other plants studied, *Apium prostratum, Goodenia stenophylla, Marianthus granulatus, Myriocephalus suffruticosus, and Velleia foliosa.* The major difference of tedera, is that it is able to germinate with high percentages without any exposure to chemical compounds.

As seeds performed well without exposure to PSC, in order of making recommendations, it would be simple to deliver normal seed without chemically imbibing them as they will produce similar germination rates when fresh seed. Carrying over seed from year to year could be a possibility on-farm, as seeds can be used without osmo-priming. In saying this, there is still the option of priming the seeds to increase germination rates. This could be applied to areas where soil available moisture is restricted by inconsistent rainfall periods that requires faster germination to make best use. There are still other factors that limit seed germination, such as scarification that need to be included to increase the benefit of PSC.

7. Germination response to moisture and saline stress

7.1 Introduction

Moisture is the essential element that seeds require for the imbibition phases and for embryo expansion and, therefore, for germination to take place. The availability of water will not necessarily germinate the seed, as this will be dictated by the dormancy status of the seed. Legume hard seeds will not germinate until this dormancy characteristic has been released by temperature or scarification (Baskin and Baskin 2004). Moisture stress can also play a large role in the induction of seed dormancy. Seeds that require cold stratification to release seed dormancy must be imbibed at the same time or dormancy will not be lost. Some seeds when imbibed with water and dried slowly, may result an increase in dormancy characteristics compared to when the seed was dried rapidly (Baskin and Baskin 1998). Seeds with differing classifications of dormancy can also be exposed to wet-dry cycles of moisture content and can be related to dormancy breaking. There are limitations on the effect of this dormancy breaking method, primarily in relation to temperature and the variations between species, so some species may have no change, decreased change or increased germination rates (Baskin and Baskin 1998). Moisture stress, or dry finishes to the growing season, at the time of ripening can also impact on the level of hardseededness of many plant species, which then can lead to seed coat impermeability (physical dormancy) (Taylor 2005).

Increased gradients of both moisture and saline stress have proven to reduce germination of legume pastures in past research (Ross and Hegarty 1980). Of the perennial legumes, lucerne has the greatest tolerance to both moisture and saline stress, however, germination percentages still decline significantly with the increased gradients (Ross and Hegarty 1980). Tedera has a larger seed size in comparison to lucerne, and a larger seed size can increase seed vigour due to greater seed storage reserves, and tolerate an increased stress gradient, particularly moisture (Benech-Arnold and Sanchez 2004).

There is significant importance to understand the limitation of germination of tedera, in both stressful moisture and saline conditions for application in WA farming systems i.e. sowing time and soil type/characteristics, and for adaptation cues to the local environment. This experiment determines the impact of water (PEG) and salt (NaCl) stress conditions on the germination of tedera seeds, in comparison with lucerne.

7.2 Materials and Methods

7.2.1 Seed collection

Seeds of tedera accessions 2 and 6 were hand harvested from the DAFWA research station in Medina (see Section 2.2.1 for more details).

7.2.2 Experimental design

Accession 2 and 6 were compared to lucerne (*Medicago sativa*) in order to gain a comparison of the germination percentage of the current major perennial legume used in WA farming systems and the experimental pasture species. Seeds for each accession were counted out into 15 lots of 60 seeds to produce 15 treatments of 3 replicates with 20 seeds per replicate.. Seeds from each group were exposed to eight PEG (polyethylene glycol) (moisture stress), and seven NaCl (sodium chloride) (saline stress) gradients. PEG gradients included, 0.00 (water), -0.25, -0.50, -0.75, -1.00, -1.50, and –2.00 Mpa. NaCl gradients included, -0.25, -0.50, -0.75, -1.00, -1.50, and –2.00 Mpa. 100 mL of each solution was placed into glass jars (NaCl) or bottles (PEG) and 8 mL distributed to the respective petri dishes with filter paper. Seeds were incubated at 20°C. All petri dishes were wrapped in plastic wrap to prevent evaporation, and covered in aluminium foil to omit light. Seeds were bleached (Appendix 10.1) with 4% sodium hyperchloride prior to plating, whereas lucerne seeds were plated without bleaching. All seeds were de-awned and cut, apart from the lucerne seed, which was left untouched. Seeds that did not germinate were exposed to tetrazolium chloride to determine viability (Appendix 10.2). All seeds and treatments were incubated from the 16th of July for 14 days.

7.2.3 Assessment of germination

Seeds were classified as germinated if there was a healthy radicle (>3 mm) present, and germinated seeds were left in the petri dishes ensuring coleoptile growth. Seeds were recorded regularly until day 14, when germination percentage and rate was calculated for each treatment.

7.2.4 Data analysis

Germination data for each replicate was calculated as a percentage of germinated to total seeds (20). Mean and standard error for each of the treatments were calculated as in Section 2.2.4. Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

7.3 Results

Germination percentage - PEG

Both accessions of tedera and the lucerne seed had negative germination responses to increasing levels of moisture (PEG) stress (Fig. 6.1). Accession 2 performed better to PEG stress levels compared to accession 6, and was similar to lucerne. All seed produced an increasing incidence of germination with a slight moisture stress gradient (-0.25 PEG), from the initial 0.00 (TC grade water) gradient. PEG treatment had a significant ($p<0.001$) effect on germination means overall. Similarly the response of accessions was significantly different ($p=0.001$). Both species declined in germination percentage after –0.50 PEG gradient. Accession 6 had zero germination at –1.00 PEG, while accession 2 and lucerne were not significantly different at any gradient. At -1.50 PEG accession 2 and lucerne also had no germination after 14 days.

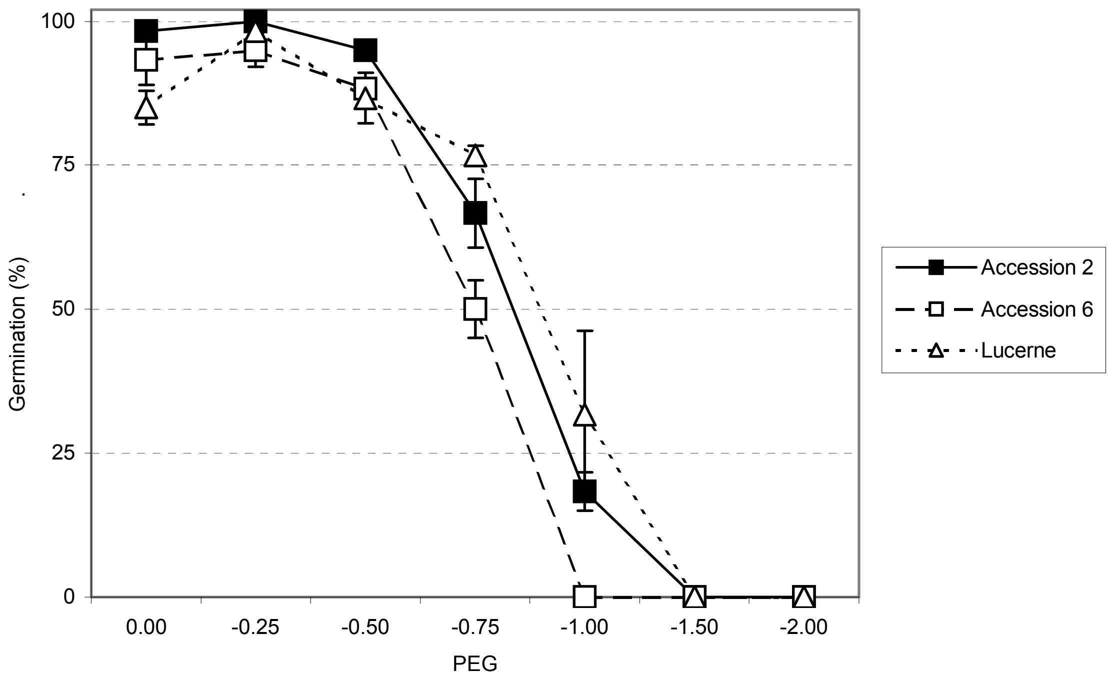


Figure 6.1. Germination response of two accessions of tedera and lucerne to moisture stress after 14 days. Bars indicate 1 s.e. of the mean.

Germination rate - PEG

Similar to germination percentages, germination rates increased slightly from 0.00 to –0.25 gradients (not significantly), and then declined thereafter. Accession 2 at –0.25 produced the highest germination percentage, at 25% germination per day (Fig. 6.2). Accession 6 and lucerne also produced their highest germination percentages at gradient –0.25, at 24% for both. Accession 2 and lucerne produced the lowest germination rates at –1.00, 2 and 3% respectively, while accession 6 had no germination at this gradient. Lucerne had the highest germination rates at the higher moisture stress gradients, but only significant at –0.75. Both accession and treatment had a significant impact ($p<0.001$) on germination rate. Accession 2 and lucerne were not significantly different compared to each other, but were significantly different to accession 6. Table 6.1 shows the honest significant differences (HSD) between treatments and accessions.

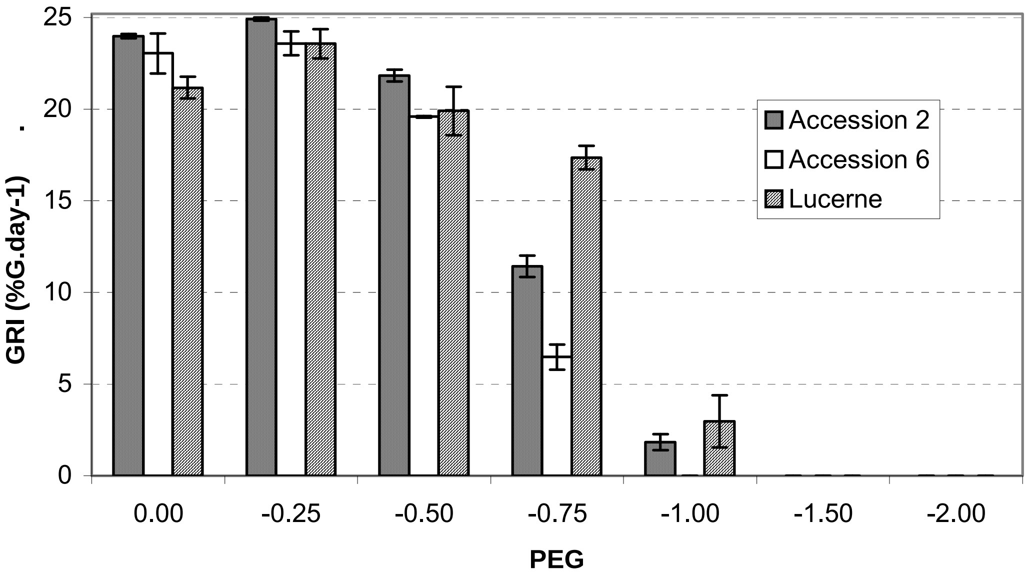


Figure 6.2. GRI response of two accessions of tedera lucerne to moisture stress after 14 days. Bars indicate 1 s.e. of the mean.

Table 6.1. Tukeys HSD of moisture stress on the germination rate of tedera.

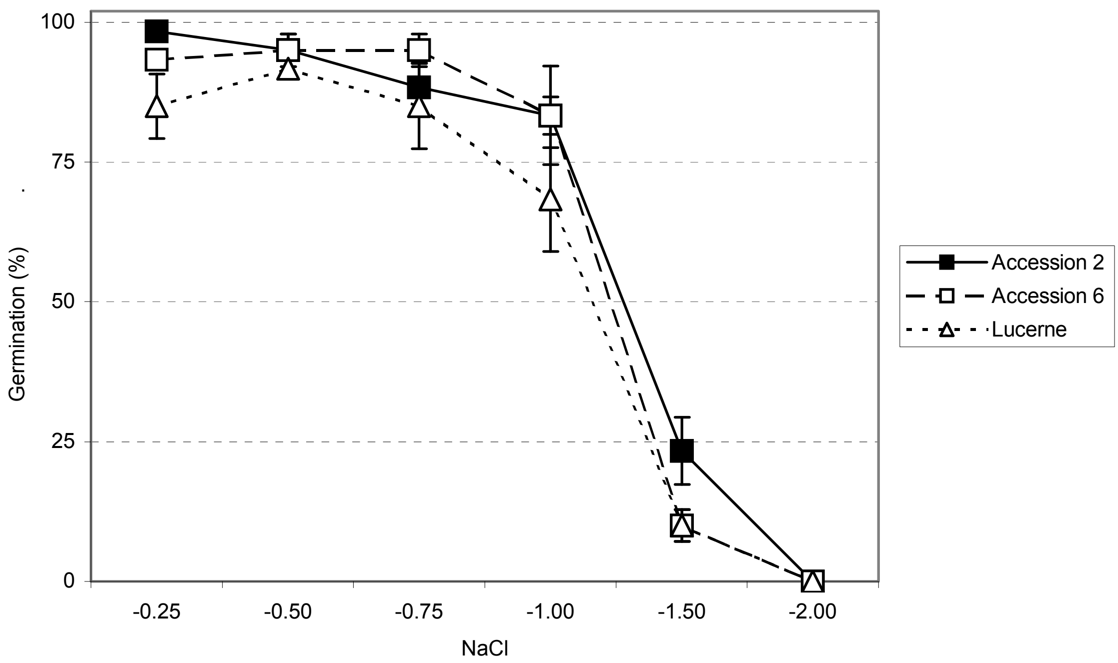
Stress - PEG			
Treatment	**Accession**	**GRI mean**	**HSD**
0.00	2	23.99	ef
0.00	6	23.04	def
0.00	Lucerne	21.17	cde
-0.25	2	24.92	f
-0.25	6	23.58	ef
-0.25	Lucerne	23.56	ef
-0.50	2	21.83	cdef
-0.50	6	19.58	bc
-0.50	Lucerne	19.90	bcd
-0.75	2	11.42	g
-0.75	6	6.48	g

-0.75	Lucerne	17.35	b
-1.00	2	1.84	a
-1.00	6	0.00	a
-1.00	Lucerne	2.96	a
-1.50	2	0.00	a
-1.50	6	0.00	a
-1.50	Lucerne	0.00	a
-2.00	2	0.00	a
-2.00	6	0.00	a
-2.00	Lucerne	0.00	a

Germination percentage - NaCl

Both tedera accessions and lucerne had good germination under saline stress, however there was a steady decline after the –0.50 NaCl gradient (Fig. 6.3). NaCl concentration had a significant difference on mean germination ($p<0.001$). Accession also had a significant difference ($p=0.014$). Rapid decline in germination percentage occurred after the –1.00 NaCl gradient, although it was only at the –2.00 NaCl gradient that there was zero germination in all seeds. Accession 2 performed slightly better than both accession 6 and lucerne. The only significant difference between accessions was accession 2 and lucerne; accession 2 performed better.

Figure 6.3. Germination response of two accessions of tedera and lucerne to saline stress after 14 days. Bars indicate 1 s.e. of the mean.



Germination rate – NaCl

Germination rates declined as the stress gradient increased (Fig. 6.4). Gradient –0.25 produced the highest germination rate for accession 2, at 24% germination per day, whereas gradient –0.50 was the highest for accession 6 and lucerne, at 24 and 22% respectively. None of the accessions were significantly different. Treatment did have a significant impact overall ($p<0.001$), however accession did not. At each gradient there were no significant differences. Table 6.2 shows the honest significant differences (HSD) between treatments and accessions.

Figure 6.4. GRI response of two accessions of tedera and lucerne to saline stress after 14 days. Bars indicate 1 s.e. of the mean.

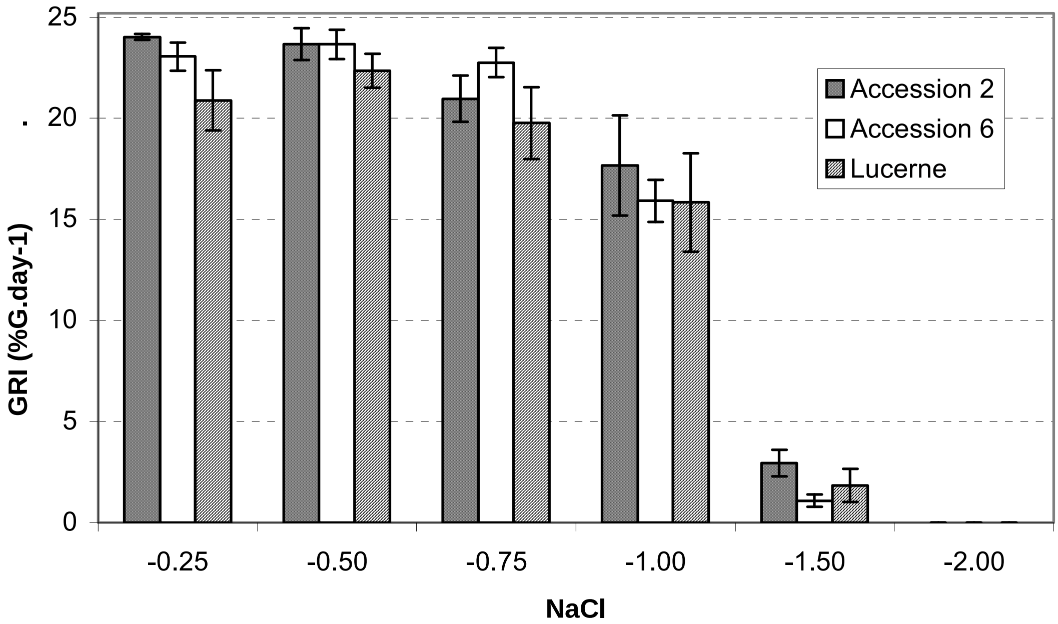


Table 6.2. Tukeys HSD of saline stress on the germination rate of tedera.

Stress - NaCl			
Treatment	**Accession**	**GRI mean**	**HSD**
-0.25	2	24.03	d
-0.25	6	23.06	cd
-0.25	Lucerne	20.89	bcd
-0.50	2	23.67	cd
-0.50	6	23.67	cd
-0.50	Lucerne	22.36	cd
-0.75	2	20.97	bcd

-0.75	6	22.76	cd
-0.75	Lucerne	19.76	bcd
-1.00	2	17.67	bc
-1.00	6	15.92	b
-1.00	Lucerne	15.84	b
-1.50	2	2.95	a
-1.50	6	1.09	a
-1.50	Lucerne	1.85	a
-2.00	2	0.00	a
-2.00	6	0.00	a
-2.00	Lucerne	0.00	a

7.4 Discussion

Moisture and saline stress both play a role in inhibiting germination. Accession 2 and lucerne performed better than accession 6 with the increasing moisture (PEG) stress gradients. Lucerne has been the primary perennial pasture with increased drought tolerance characteristics that make it suitable to a wide range of WA environments. Lucerne is regarded to have very high drought tolerance, and in comparison to birdsfoot trefoil and white clover, which have moderate and low drought tolerance (Moore et al. 2006). This experiment shows that tedera has a similar ability to lucerne, increasing its potential suitability to the WA Mediterranean-type environment. The germination rates responded similarly to that of final germination percentages.

Saline (NaCl) stress had similar results to that of moisture stress. Germination percentages and rates declined as the gradient increased. None of the accessions or lucerne were significantly different at any of the gradients, and all had germination until the highest gradient of –2.00. Germination rates were not significantly different between accessions or lucerne at any gradient. The most important information from this is that tedera can produce the same final germination percentages, and germination rates as lucerne under saline stress conditions. Lucerne is also known as one of the most tolerant perennial species to saline soils in WA with moderate tolerance to saline conditions compared to other perennial speices such as birdsfoot trefoil and white clover which have nil, and low tolerance to salt (Moore et al. 2006). Tedera has the potential to survive in the same conditions as lucerne.

Tedera has the ability to perform just as well as lucerne under both moisture deficit and saline conditions, two characteristics that are extremely important for the WA Mediterranean environment. Accession 2 also performed slightly better than accession 6 in both experiments showing a genotype effect. More research is required on this aspect to fully understand the differences in the field. Tedera did not improve on any of these characteristics compared to lucerne, however there is potential for tedera to outperform lucerne in other aspects, such as agronomic factors like sowing depth, which will be studied, but also establishment and survival characteristics, where further research will be needed.

8. Effect of sowing depth on germination and emergence

8.1 Introduction

Sowing depth is a key aspect in the germination and establishment potential of a plant species in any environment. Species such as lucerne must be sown at 5-10 mm for best establishment (Moore et al. 2006). If lucerne is sown too deep there is potential for reduced seedling emergence, and therefore poor establishment (Table 7.1). Similarly, if the seed is surface sown, there is a higher risk of poor establishment due to limited moisture availability on the surface (Moore et al. 2006). All plant species, such as wheat (*Triticum aestivum*) in conventional sowing practices must be sown under the surface to take advantage of soil moisture reserves, and therefore effective establishment. This has subsequent effects on yield (Kirby 1993). There is a significant difference in seed size between tedera and lucerne, so there is the potential for increased seedling emergence from a greater depth, as seed size has an important influence on the seed's ability to emerge from depth (Forbes and Watson 1992). This experiment focused on a wide range of depths, from surface sowing, to 10 cm in depth.

Understanding sowing depth requirements for tedera will allow recommendations to be made about the efficacy of sowing with conventional tillage. Traditional sowing equipment can be inaccurate when trying to achieve a uniform sowing depth, so the influence of range in sowing depth of tedera will be important in making recommendations for using broadacre equipment for sowing. In this experiment, the effect of sowing depth on germination percentage of tedera was studied.

Table 7.1. Effect of sowing depth on % emergence of lucerne. (Sund et al. 1968).

Depth	Sand	Loam	Clay
(cm)			
1.25	71	59	52
2.50	73	55	48
3.75	55	31	28
5.00	40	16	13

8.2 Materials and Methods

8.2.1 Seeds

Seeds of tedera accessions 2 and 6 were hand harvested from the DAFWA research station in Medina (see Section 2.2.1 for more details).

8.2.2 Experimental design

Seeds from accession 2 were counted out into 6 lots of 60 seeds; 6 depths x 3 replicates x 20 seeds per replicate. The six depths were, 0, 2, 4, 6, 8, and 10 cm below the surface. These depths were chosen as they represented the depth of sowing for pasture and annual crop plants. There was an expectation that tedera would not germinate from 10 cm in depth, as lucerne does not. The soil type used was clean, coarse yellow builder's sand, pH 6.8 (CaCl). The sand was sourced from Naval Base Sand Supplies. Seeds were planted into polystyrene boxes (Fig. 7.1) for ease of handling and recording. The boxes (535L x 347W x 305D) contained a 4 cm layer of potting mix (Waldecks) on the bottom, to aid drainage, with 15 cm of coarse yellow sand placed on top (Fig 7.2). Five holes, 1 cm in diameter, were drilled into the bottom of each box prior to sowing to drain excess water. Seeds were sown in rows spaced 7.65 cm, with a distance between seeds of 1.65 cm. All seeds were de-awned and cut to maximise potential germination, as this treatment had the uppermost germination percentage in Chapter 3 and 4. Seeds were bleached prior to planting with a 4% sodium hyperchloride solution. Each box contained a replicate of treatments, arranged in a randomised block design. The experiment was totally randomised to prevent any biased results in the boxes, such as edge effects or the influence of adjacent treatments. Seeds were sown into holes made with a metal rod of 5 mm diameter, marked off to the appropriate depths. These holes were then covered to the surface with fresh sand. Boxes were placed in a glasshouse at South Perth, and watered regularly, so as the soil surface remained moist. Hourly temperatures were recorded with an electronic temperature button (Fig. 7.3) placed on the soil surface. Minimum, maximum, and average temperatures were calculated.

Figure 7.1. Polystyrene box design.

Figure 7.2. Cross-section of soil pit.

8.2.3 Assessment of germination and emergence

Seeds sown at depths of 2, 4, 6, 8, and 10 cm were recorded as having emerged once a coleoptile and cotyledons had emerged from the soil surface. Seeds sown on the surface were given a tentative germination classification if they had a healthy radicle protruding (>3 mm). These seeds were then classified as having emerged once a coleoptile was developed. Germination and emergence were scored weekly for 39 days. Final emergence percentage and rate were calculated at day 39.

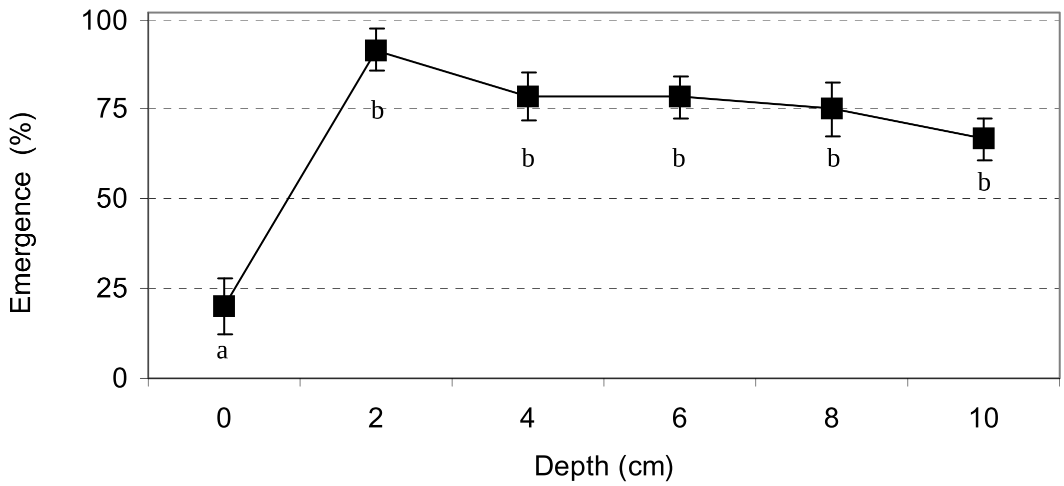
8.2.4 Data analysis

Germination and emergence data for each replicate was calculated as a percentage of emerged to total seeds. Mean and standard error for each of the treatments were calculated as in Section 2.2.4. Statistical tests for significant differences were calculated using analysis of variance (ANOVA) in Genstat, and means were compared using Tukeys HSD (Honestly Significant Difference). Germination rate index (GRI) was calculated in excel spreadsheets, by germination over time.

8.3 Results

Emergence percentage

Sowing depth had a significant effect on emergence ($p<0.001$). Seeds sown at 2 cm had the highest total emergence after 39 days of 92%, followed by 4 and 6 cm (both 78%), 8 cm and 10 cm depth (Fig.7.4). Seeds sown on the surface had the lowest total emergence of 20%. Mean germination total for surface-sown seed was 95%, but by day 39 most of these seedlings had died without cotyledons emerging from the fruit. There was no significant difference between the depths, apart from seeds sown on the surface.



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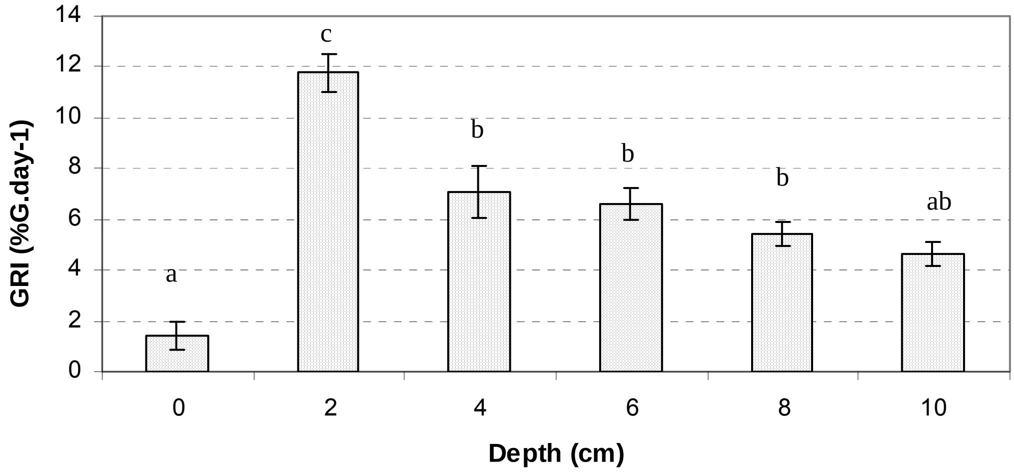
Emergence rate

Sowing depth had a significant effect on GRI ($p<0.001$). Emergence rate was the highest from a depth of 2cm, at 12% emergence per day (Fig. 7.5). Emergence rates declined with an increase in depth to 10cm. Seeds that were surface sown had the lowest emergence rate, at 1% per day, due to the high death rate of germinated seeds.



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8.4 Discussion

Tedera has a versatile sowing depth as it can emerge, at high rates (>75%), from 8 cm, and still >65% from 10 cm. It was unexpected that tedera would even germinate from 10 cm in depth, as most other pastures, such as lucerne must be sown at a depth of 5-10 mm for effective establishment (Moore et al. 2006). Figure 7.6 shows the seedlings that emerged from 0 through 10 cm in depth. The hypocotyl shows the depth of sowing from roots to cotyledons. The benefit of this is that tedera can be sown effectively with a conventional tillage machine, similar to what current annual crops are sown with. The equipment is not necessarily accurate in regard to depth accuracy, therefore a larger leeway from 2 to 8 cm where significant germination rates are possible, will benefit growers. This is a substantial agronomic benefit over other perennial and even annual species. Surface sowing would not be recommended, as emergence percentages are very low.

Figure 7.6. Tedera seedlings, showing sowing depth from 0 through to 10 cm.

This experiment was conducted in only one soil type, and as studies by Sund et al. (1968) showed, soil types had a significant effect on emergence percentages in lucerne. It would be expected that tedera would produce similar results to this, however, further research is required. Soil moisture availability also varies between soil types. The sand used in this experiment had high drainage, an important characteristic of sand (Moore 2001), whereas other soil types such as clay are recognised to have increased moisture-holding capacities, and different soil structure (Moore 2001), which can have subsequent time of sowing benefits. As mentioned in Chapter 2, the best time for sowing tedera in WA would be from late Autumn to late Winter when water is non-limiting to minimise the effect of soil type. There are numerous other factors that can impact on tedera germination in the field, such as factors mentioned in Section 1.1.3; acidity and waterlogging. These all need to be studied further.

9. An establishment package for tedera

The information gained in this research can be used to develop guidelines for tedera sowing and establishment. The key steps for establishment of tedera are outlined below.

1. Select the paddock for sowing

Plan a year ahead of sowing, if possible, and reduce seed set of annual weeds in spring by grazing and spray-topping

2. Use good quality seed

Use recently harvested seed where possible. Seed harvested in early-mid summer should not have significant dormancy issues for sowing in late autumn or winter. Seed longevity studies have not been conducted, but seed older than two years should be germination tested to ensure viability.

3. Prepare a weed free seed bed prior to sowing

Control summer weeds and germinating weed seedlings at the break of season with knockdown herbicides or cultivation to prepare a weed-free seed bed. Paddock preparation for tedera should use similar good agronomic practice as for sowing crops or other pasture species.

4. Sow into a moist seedbed in autumn or early winter

Tedera is best suited to sowing in late autumn or early spring – a similar time to annual pastures and crops. It should be sown into moist soil. The option of dry sowing has not been tested, but it is likely to be more risky.

5. Inoculate with the appropriate rhizobium strain

If tedera is commercialized, it is likely to need its own special rhizobium inoculant to fix nitrogen. Either lime pellet the seed or add granular inoculants to the seed box (if available). Rhizobia for tedera are under development.

6. Drill seed in with conventional seeding machinery

Conventional seeding machinery can be used to sow tedera. Its relatively, large, robust seed means that particular care is not needed to ensure sowing depth is precise. Sowing depth should be within the range of 2-8 cm. Some soil cover is required, so do not drop seeds onto the soil surface.

Sowing into furrows is important to scalp away non-wetting sand, remove weed seeds from near the tedera seedlings and harvest rainfall. Furrows capture rainfall and increase seedling survival, particularly in dry periods soon after sowing. They act to turn relatively small rainfall events into useful soil moisture for growth and survival. For example, 3-5 mm of rain can effectively become 10-15 mm for seedlings in the bottom of a furrow.

Press wheels could be used to provide good seed contact with soil moisture in the furrow bottoms.

7. Control weeds and pests (insects, kangaroos and rabbits) post-sowing

Pests of other pasture legume species should be controlled, particularly in the first few weeks after sowing. Particular attention should be given to controlling redlegged earth mite, aphids, lucerne flea and caterpillars.

Grass selective herbicides are likely to be useful for controlling grassy weeds. Little is known at this stage about the most appropriate herbicides to use for post-emergent control of broadleaf weeds. Good pre-emergent weed control is a key strategy. This is best done by commencing the year before sowing, by reducing seed set of annual weeds in spring, and killing germinating weeds seedlings at the break of season before sowing.

8. Defer grazing until plants are well-rooted

Optimum grazing management strategies for tedera have yet to be devised. However, grazing should not commence until seedlings are firmly anchored and cannot be readily pulled up by hand. This is particularly important for a perennial species like tedera, as the opportunities to recruit new seedlings from seed set are likely to be limited, so long-term plant density will be related to the density of plants that successfully establish.

This package can only be regarded as preliminary, but it provides a framework for agronomic studies to further refine optimum establishment conditions for tedera.

10. Acknowledgements

This research was conducted in 2009 by Mr Cale Beard as an Honours project through Curtin University of Technology, as part of the Future Farm Industries CRC project "*Reliable establishment of non-traditional perennial pasture species*", with additional funding from Meat & Livestock Australia, Australian Wool Innovation Ltd and the former Land and Water Australia. The project was supervised by Dr Pippa Michael (Curtin University of Technology), with technical co-supervision by Dr Phillip Nichols (Department of Agriculture and Food Western Australia, DAFWA) and Dr Christopher Loo (Kings Park Laboratories and The University of Western Australia). This work was also conducted as part of a DAFWA undergraduate studentship program.

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GOVERNMENT OF WESTERN AUSTRALIA
Department of Agriculture and Food

Bulletin 4275
November 2009
ISSN 1833-7236

Identifying sub-tropical grass seedlings

FUTURE FARM INDUSTRIES CRC

Acknowledgements
Peter Maloney (DAFWA Image Resource Centre) took most of the photos and prepared them for publication. This farmnote was produced as part of the project 'Reliable establishment of non-traditional perennial pasture species' with funding from the Future Farm Industries Cooperative Research Centre, Meat and Livestock Australia, Australian Wool Innovation Ltd. and Land and Water Australia.

Department of
Agriculture and Food
GOVERNMENT OF
WESTERN AUSTRALIA

Bulletin 4275
November 2009
ISSN 1833-7236

Identifying sub-tropical grass seedlings

Brad Wintle, Geoff Moore and Phil Nichols
(Pasture Science, DAFWA and Future Farm Industries CRC)

There is considerable interest in growing sub-tropical (or warm season) perennial grasses for out-of-season green feed, especially on the south coast and in the northern agricultural region (NAR). They consist of two types: creeping grasses, which spread by either above ground runners (stolons) or below ground runners (rhizomes) and bunch grasses, which are tufted and do not spread by runners.

The main species sown include Panic grass, Rhodes grass, Kikuyu, Setaria, Signal grass, Digit grass, and Bambatsi panic and are often sown as a mixture, comprising 2 or more species. This bulletin describes how to identify these grasses at the seedling stage.

Panic grass

(*Panicum maximum* syn. *Megathyrsus maximus*),
cv. Gatton and Green panic (cv. Petrie)

Panic grass is a leafy, bunch grass with good palatability and feed quality. It has performed well on sandy soils in the NAR and in field trials on the south coast.

Seedlings may be identified by:

- leaf blades that are flat and slightly shiny with a smooth surface, often in pairs opposite each other on the stem
- generally semi-erect to erect growth habit, tillers radiating from the crown at about 45° from the ground
- good early vigour (see Table 1)

Variety differences:

Gatton panic—dark green, hairless leaves with more pronounced mid-rib and red-purple pigmentation on smooth, lower stems.

Green panic—distinctive light-green foliage (almost appearing N deficient), some hairs on lower leaves and stems, green stems (no red-purple pigmentation).


2.5 cm


Red-purple stems radiating from crown at about 45°

Rhodes grass

(*Chloris gayana*)

cvs. Katambora, Pioneer, Finecut⌽, Topcut⌽ (diploids) and Callide (tetraploid)

Rhodes grass is a creeping perennial which spreads through stolons. It is adapted to a wide range of soil and climatic conditions and has moderate to high drought tolerance.

Seedlings may be identified by:

- the first leaf pair being short and broad
- leaf blades that are flat and distinctively curved downwards, with a slight twist
- no hairs on stems and leaves, but sparse, 2-3 mm long hairs where the leaf blade meets the stem (ligule)
- semi-erect growth habit, which becomes more prostrate as they tiller and develop stolons
- base of stem often with red-purple pigmentation
- vigorous early growth (most vigorous of the sub–tropical grasses – see Table 1)


3.1 cm


Leaf blades
curve downwards
and twist

Kikuyu

(*Pennisetum clandestinum*)
cv. Whittet

Kikuyu is a creeping grass which forms a dense turf from both stolons and rhizomes. It has moderate waterlogging tolerance and is well adapted to the south and west coasts, where it has been extensively sown. Kikuyu will persist in the NAR, but biomass production is poor if subsoil moisture is low.

Seedlings may be identified by:

- long, narrow, slightly folded, light-green leaves with a prominent mid-rib
- semi-erect growth, with short inter-nodes between leaves which are usually opposite each other
- base of the stem being white to pale green, while larger seedlings have fleshy, white stolons
- good early vigour (see Table 1)


Long narrow, v-shaped leaf blades
3.2 cm


White, fleshy stolons

Setaria

(*Setaria sphacelata, S. splendida*)
cvs. Narok, Solander, SplendaΦ

Setaria is a moderate to tall bunch grass with comparatively good cool season growth and moderate waterlogging tolerance. It has performed well in trials on the south coast (>475 mm) and has potential on waterlogged soils in the NAR.

Seedlings may be identified by:

- lower stems that are distinctively flattened
- stems and leaves with a distinct smooth appearance
- leaf blades that are light-green and slightly folded with a mid-rib, while new leaves may have a crinkly appearance
- base of stems with red-purple pigmentation sometimes present
- moderate early vigour (less vigorous than panic and Rhodes grass)


2.4 cm


Flattened,
smooth stem


Crinkly
appearance on
new leaves

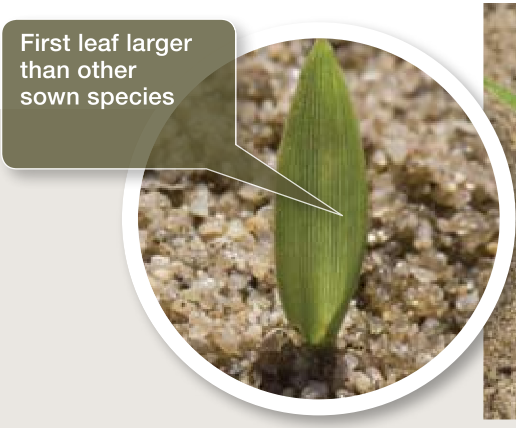
Signal grass

(*Urochloa brachiaria*)
cv. Basilisk (usually sold as Signal grass)

Signal grass is a creeping grass which can form a sward, but under WA conditions behaves as a bunch grass. It has some potential on sandy soils in areas with mild winters and a low incidence of frosts in the NAR, but is generally not suited to the south coast.

Seedlings may be identified by:

- distinctive shiny, lime to bright green leaves
- leaf blades that are flat to slightly v-shaped, with larger seedlings developing a clear mid-rib
- emerging leaf blades that are distinctively rolled
- erect to semi-erect growth habit with a few large leaves arranged alternately on the stem with long inter-nodes between leaves
- good early vigour (see Table 1)


First leaf larger than other sown species


4.5 cm


Large, lime to bright green leaves


Emerging leaf blades rolled

Digit grass

(*Digitaria eriantha*)
cv. Premier

Premier digit grass is a highly palatable bunch grass. It has not been widely evaluated in WA, but in northern NSW digit grass has persisted and performed well on a wide range of soils and environments.

Seedlings may be identified by:

- dull green, broad and slightly curved leaves;
- conspicuously hairy lower stems
- leaves that are hairless, except for a few hairs near the base
- tillering from small plants, giving them a compact 'chunky' appearance
- slow early growth (much less vigorous than panic and Rhodes grass – see Table 1)


Base of stem
is hairy
1.0 cm

Bambatsi panic

(*Panicum coloratum*)
cv. Bambatsi

Bambatsi is well adapted to fine-textured soils (e.g. cracking clays) and has low biomass production on sandy soils, even though it may persist.

Small seedlings have few distinguishing features, compared to mature plants. Its main seedling features are:

- dull green leaves with a flat leaf blade; which become blue-grey in mature plants
- no obvious mid-rib when small, while larger seedlings develop a prominent white mid-rib
- stems and leaves with few or no hairs
- slow early growth (slowest of all the sub-tropical grasses – see Table 1)


0.8 cm


Prominent
white mid-rib

Table 1. Comparative seedling size of sub-tropical grasses 18 days from an early March sowing in Perth. Seedlings were grown under irrigation and measurements are the average of 6 random plants.

SPECIES	AVERAGE HEIGHT (cm)	AVERAGE WIDTH (cm)
Panic grass	2.2	2.2
Rhodes grass	3.5	2.1
Kikuyu	3.5	3.5
Signal grass	2.2	3.5
Digit grass	1.0	1.6
Bambatsi panic	0.5	1.3

Establishment guide for sub-tropical grasses – key steps to success

Geoff Moore, Ron Yates, Phil Nichols, Brad Wintle and John Titterington (Department of Agriculture and Food Western Australia), Phil Barrett-Lennard (agVivo and Evergreen Farming) and Chris Loo (University of Western Australia and Kings Park and Botanic Garden)

Acknowledgements

Funding for the project, ‘Reliable establishment of non-traditional perennial pasture species’ was provided by DAFWA and the Future Farm Industries CRC (FFI CRC), together with Meat & Livestock Australia, Australian Wool Innovation Ltd and the former Land & Water Australia. The machinery case studies were funded through the DAFWA – FFI CRC project, ‘Productive, persistent tropical grasses in farming systems’.

We acknowledge the support and encouragement of Evergreen Farming, whose members have led the early adoption of sub-tropical grasses in WA. We would like to thank the following producers: Bob Hendrie (Gillingarra), Quentin Fabling (Eneabba) and Rod O’Bree (Kojarena) for providing land for field trials and for assisting with site management and at field days. The assistance of Grant Bain and Morgan Sounness is gratefully acknowledged for their valuable input at project planning workshops. Special thanks to Ken Hodby, Dan Rieusset, Morgan and Debbie Sounness, Peter Summers and Bob and Anne Wilson for sharing their experience and knowledge in the machinery and establishment case studies.

Tim Wiley (formerly DAFWA) was a leading advocate for the use of sub-tropical grasses in the Northern Agricultural Region of WA from the early 1990s until 2009 and provided valuable input at project planning workshops and assisted with identifying field sites.

Contents

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1. Summary - key steps for establishment

The following ten points summarise the key steps for successful establishment. To ensure a productive long-term pasture aim for a perennial grass density of at least 10 established plants/m^2 in the autumn after sowing.

- Plan a year ahead and reduce weed seed set, commence control of rabbits and kangaroos and consider sowing a cereal to provide stubble for reduced erosion risk
- Purchase good quality seed of appropriate species and varieties
- Control weeds and insects prior to sowing
- Sow into moisture in late winter to early spring (depending on district) - if soil moisture is limiting defer sowing until the following year
- Set up the seeder to sow into furrows with trailing press wheels and a row spacing of 50-60 cm
- Sow 2-5 kg/ha of seed, depending on seed quality and whether coated or uncoated
- Sow at a depth of 5-10 mm
- Don't sow too fast
- Control weeds and pests (insects, kangaroos and rabbits) post sowing
- Defer grazing until the grasses are well established

2. Introduction

Sub-tropical perennial grasses are now being widely sown in the Northern Agricultural Region (NAR) and on the south coast of Western Australia (WA). Since 2000 the area sown to perennial grasses in the NAR is estimated to be more than 50,000 ha, while there are approximately 150,000 ha on the south coast (mainly kikuyu).

Perennial grasses have multiple production and natural resource management (NRM) benefits. Animal production benefits come through increased productivity of poor sandy soils, lengthening of the growing season in both autumn and late spring - summer, and provision of green feed outside the growing season. NRM benefits are provided by a reduction in the potential for wind erosion over summer and mitigation of the effects of dryland salinity.

Sub-tropical grasses are transforming productivity on deep sands in both the Northern Agricultural Region and South Coast of WA

Five years ago establishment of perennial grasses at the paddock scale was typically patchy, with many areas of poor perennial plant density. Seeding failures were also common. However, a few farmers were having success with their seeding systems, which showed that successful establishment was possible. Establishment success of sub-tropical grasses overall has improved dramatically since then. A major contributing factor has been a greater understanding of their seed biology and agronomic requirements, which has resulted in the development of an establishment package for sub-tropical grasses.

Rapid adoption of the key elements of the package has resulted in the 'bar' being raised considerably throughout the industry and farmers now expect a much higher and more even plant establishment. Leading farmers are now regularly achieving uniform establishment across the paddock each year, in spite of variable seasonal conditions. The contributions of innovative farmers from Evergreen Farming, the Mingenew-Irwin Group and the West Midlands Group, in addition to the Northern Agricultural Catchment Council and South Coast NRM, have been a fundamental part of this improvement.

This bulletin describes the steps for successful establishment of sub-tropical perennial grasses. It describes each of these steps in detail and provides results from research and development to support the recommendations. This is followed by a series of case studies from leading farmers. The bulletin is applicable for a wide range of species, including Rhodes grass, panic grass, kikuyu, signal grass, setaria, digit grass and bambatsi panic, either sown alone or in mixtures. It is primarily aimed at the NAR and south coast regions of WA, but is also applicable to other areas of Australia with winter-dominant rainfall climates that are not prone to frosts.

Grant Bain from Walkaway led the way, showing that good, even establishment of sub-tropical grasses was possible (photo 2004)

3. Planning ahead

For best results, plan a year ahead of sowing and assess the paddock for weed burden, risk of wind erosion and potential pest problems.

Reduce weed seed-set by grazing and spray-topping, especially for weeds that can be difficult to control, such as silver grass, annual ryegrass, erodium, ice plant and wild radish.

In exposed, sandy paddocks that are highly susceptible to wind erosion, consider sowing a cereal crop in the year prior to establishing sub-tropical grasses. The grasses can then be sown into the standing cereal stubble, which reduces the potential for soil erosion while they are establishing.

Rabbits and kangaroos can severely damage perennial grass seedlings. If they are likely to cause problems, commence their control the year before sowing.

Consider purchasing seed of Panic grass, signal grass and setaria the year before seeding to overcome post-harvest dormancy. This is described in more detail in Section 5.1. The other grasses do not have such dormancy.

Consider sowing companion annual legumes in the year before sowing the sub-tropical grasses to provide a source of nitrogen and to increase winter production of the paddock. This is described in more detail in Section 16.

4. Species selection

Select species and varieties suitable to the climate and soils of the paddock to be sown. Table 1 outlines suitable species for different soil types in both the NAR and south coast. DAFWA Farmnote 445 provides more details on variety selection for the NAR, while DAFWA Farmnote 446 provides this information for the south coast.

In the NAR panic and Rhodes grass have been the main species sown and are usually sown together in a mix. Signal grass has often been included in the mix for non-wetting sands, as its larger seeds and ability to germinate from depth act as an insurance against establishment failure from sowing too deep or following sand in-fill from strong winds after seeding. However, preliminary data suggests that signal grass has high concentrations of saponins (Spadek, Ewald and Moore, unpublished data), which can cause secondary photosensitisation, so caution is needed before considering sowing this grass.

On the south coast kikuyu is widely sown, while panic grass, setaria and Rhodes grass are also well adapted.

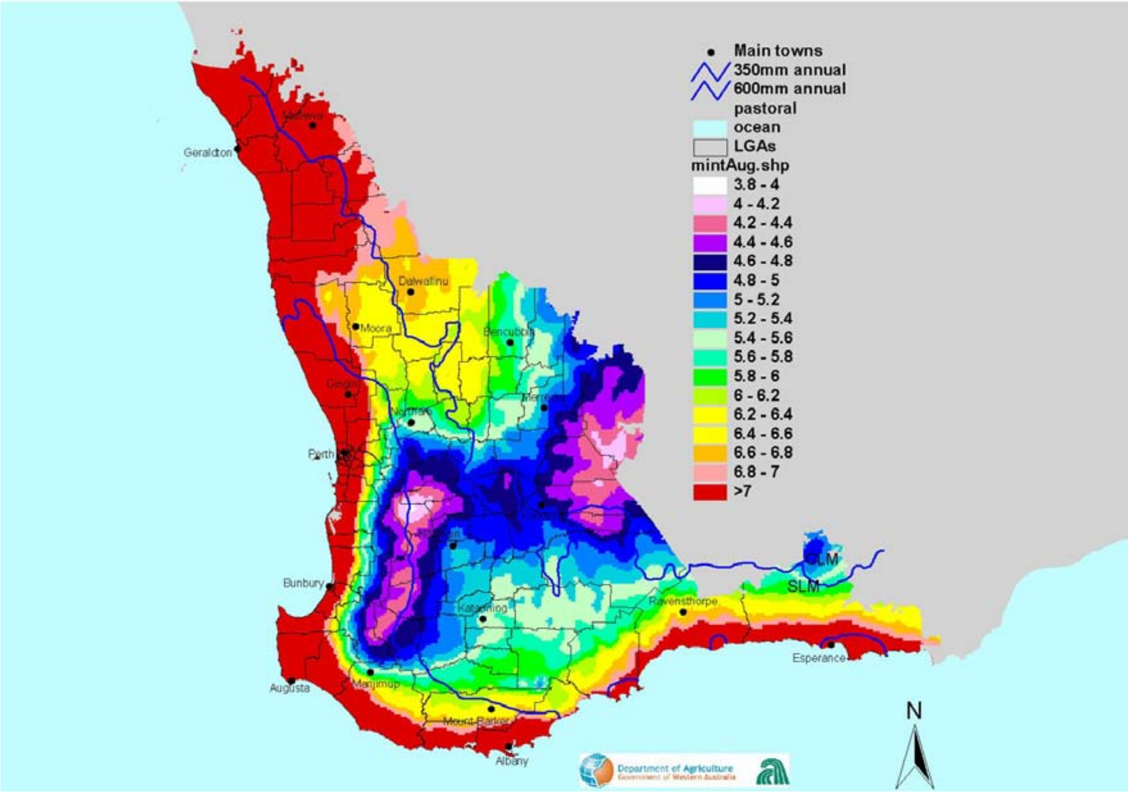


Figure 1. Map of south-western Australia showing August mean minimum temperatures (^{o}C). The adaptation zone of sub-tropical grasses is approximated by the areas shaded in red

Table 1. Soil and climate requirements and suitability of sub-tropical grasses for the northern agricultural region (NAR) and the south coast of Western Australia (adapted from 'Perennial pastures for Western Australia' DAFWA Bulletin No. 4690)

Species	**Drought tolerance**	**Frost tolerance**	**Soil type**	**Minimum soil pH_{Ca}**	**Waterlogging tolerance**	**Suitability to south coast**	**Suitability to NAR**
Bambatsi panic	High to very high	Low	Fine-textured clays	5.0	Moderate	Niche (>325 mm)	Niche (>375 mm)
Consol lovegrass	High to very high	Moderate	Range including coarse-textured soils	4.0	Low to moderate	Moderately well adapted* (>350 mm)	Moderately well adapted* (>375 mm)
Digit grass	Moderate to high	Low to moderate	Range except deep pale sands	4.2	Low	Moderately well adapted (>400 mm)	Moderately well adapted (>450 mm)
Kikuyu	Moderate to high	Moderate (but re-grows from rhizomes)	Range including deep sands	4.0	Moderate	Well adapted (>400 mm)	Niche (>500 mm)
Panic grass	High to very high	Low	Range including pale deep sands	4.3	Low	Well adapted (>350 mm)	Well adapted (>325 mm)
Rhodes grass	High	Low	Range including deep sands	4.3	Moderate	Well adapted (>400 mm)	Well adapted (>425 mm)
Setaria	Moderate	Low to moderate	Range, especially duplex soils	5.0	Good	Well adapted (>475 mm)	Niche (>550 mm)
Signal grass	Moderate to	Sensitive	Sands	4.0	Low to	Not suitable	Moderately well

	high				moderate		adapted** (>450 mm)

* Widely adapted but lower feed quality and palatability

** Preliminary results suggest signal grass has high concentrations of saponins (Spadek, Ewald and Moore, unpublished data)

5. Seed Quality

Quality of sub-tropical grass seed lots can vary widely in terms of germination, purity, dormancy and weed seeds. There are no national standards for seed quality, so it can be a case of 'buyer beware'.

The germination of most sub-tropical perennial grass seed lots can vary from 10% to more than 60%, but is rarely higher than 70%. This compares with annual pasture legumes and grasses where the germination is usually above 80%. An exception is kikuyu, which normally has germination >80%.

Commercial seed batches often contain empty florets, immature seed, dormant seed and dead seed, in addition to viable seed. Some seed merchants use the term *pure live seed* when referring to seed quality (see box).

The grasses can be sown alone or in mixtures (some seed merchants supply ready-made sub-tropical grass mixtures). Read the seed label carefully and understand the proportion of each species or variety in the mix (% weight) and their germination %.

For species with post-harvest seed dormancy (panic grass, setaria and signal grass) consider purchasing seed the year prior to sowing (see below).

5.1. Post-harvest seed dormancy

Following harvest, seed of panic grass, setaria and signal grass displays a period of post-harvest dormancy. In this state seeds are viable but have low germination. After a period of storage germination improves. The time required to reach acceptable germination levels varies between species and storage conditions (Figure X).

In general, setaria seeds require 4-5 months storage after harvest to reach acceptable levels of germination, while 'Green' and 'Gatton' panic require 8-10 months storage to achieve maximum germination. Signal grass also has a long period of after-ripening dormancy and may take 9-12 months to reach maximum germination. Rhodes grass, digit grass and kikuyu exhibit little or no seed dormancy.

Seed dormancy is a particular issue for sub-tropical grass seed coming into WA from northern Queensland, where most of the seed production in Australia is located. The time from harvest (January to April) to the recommended sowing time in WA of August – September may be insufficient for the seed to reach acceptable levels of germination. This differs from the situation in sub-tropical areas of eastern Australia, where sowing does not generally occur until November – February, by which time post-harvest seed dormancy has largely broken down.

High levels of seed dormancy at sowing present a problem for two reasons. Firstly, establishment is likely to be lower than expected for a given seeding rate. Secondly, seeds that later come out of dormancy and germinate after summer rain are more likely to perish from moisture stress before they can establish.

5.2. Checking seed quality

Check the seed label on the bag carefully. The term 'fresh seed' equals dormant seed. Low germination equates to poor quality seed, unless there is a high proportion of fresh (dormant) seed. Always compare seed price on the cost per kg of viable seed (see 'Pure Live Seed').

If there is a large amount of fresh seed request a copy of the Seed Analysis statement from the seller. This analysis is usually undertaken within 2 to 4 weeks of harvest; this allows an estimation of harvest date.

If the time from the estimated harvest date is less than 6 - 8 months, then post-harvest seed dormancy of panic grass, signal grass and setaria is likely to be high. In this case sowing rate needs to be increased or the seeds should be stored for sowing in the following year, when dormancy will have subsided.

If the time from estimated harvest date to seeding is more than 8 - 10 months then seed dormancy should be low. Storage conditions can affect seed quality, so it is preferable to re-test the germination if the seed analysis statement is more than 12 months old. Consider purchasing panic grass, setaria and signal grass the year before seeding and store under dry conditions. Refer to 'Seed storage and its effect on germination'.

If in doubt about the quality of a seed lot obtain a current germination test result through an accredited seed testing laboratory (e.g. AgWest Plant Laboratories). A home germination test can be done to get an approximate idea of germination (see box).

Home germination test

To conduct a simple test to give an approximate germination of your seed lot, all you need is a plastic container with lid (e.g. takeaway food container) and some paper towels. This should be done 2-4 weeks prior to the intended sowing date.

- Place three layers of paper towelling in the bottom of the container, wet with water and pour off any free surface water
- Sprinkle ~200 seeds across the surface
- Close the lid tightly to minimise moisture loss
- Place the container in a warm location (>20°C)
- Check regularly to ensure the paper towelling is moist and carefully re-moisten as required.
- Count germinating seedlings after 14 days.
- Assess germination as poor (<10%), fair (15-30%) or good (>35%), where:

Germination % = (no. germinated seeds) / (total no. seeds) x 100

If assessing a seed mix, determine the germination of each species in the mix (see photo to identify seeds).

5.3. Coated seed and mixes

Sub-tropical grass seeds are often sold with brightly coloured coatings, which consist of polymers that pelletise the seed and bind together a range of chemicals, such as fungicides, insecticides, nutrients, seed primers and lime.

Coated seeds have several advantages over naked seeds. They are easier to handle and flow more easily through seeding machinery; this is particularly the case with fluffy-seeded species, such as Rhodes grass and digit grass. By comparison, uncoated seeds of these species need to be mixed with a carrier, such as fertiliser, to enable seed flow through machinery. The bright colour of the seed coating also enables easy checking of seed placement.

The main disadvantage of seed coatings is that they markedly increase seed size, which means a large reduction in the number of seeds per kilogram. Data from a New South Wales study found coated seeds of Bambatsi panic grass and Premier digit grass were 4-5 times heavier than uncoated seeds, while coated seeds of Katambora Rhodes grass were up to 12 times heavier (G. Lodge, unpublished data). In these examples, it means only 1 kg of actual seed in every 4-5 kg of Bambatsi or Premier seed lot and only 1 kg of actual seed in every 12 kg of Katambora Rhodes grass seed lot. Furthermore, coated seed lots can often hide the presence of other seeds, empty florets, straw and other debris in the sample.

For coated seed, sow at the higher recommended rates to compensate for the lower number of viable seeds per kilogram.

Some commercial seed coats contain *priming agents*, which are chemicals aimed at reducing seed dormancy. However, independent testing by King’s Park has demonstrated that while these priming agents can sometimes be effective at overcoming dormancy in the laboratory or glasshouse, their effect is not reliable under field conditions. While seed coatings often contain fungicides, insecticides and nutrients, their effectiveness at delivering these benefits to establishing seedlings has not been independently tested.

Seed of the sub-tropical grass species used in Western Australia

Pure Live Seed

Pure Live Seed (PLS) is a measure of seed quality of a seed lot used by some seed companies and re-sellers and is expressed as a number between 0 and 1.

PLS is calculated by multiplying the % purity by the % germination and then dividing by 10,000. Here purity refers to the % of pure seed in the seed lot; it does not include empty florets or other seeds.

PLS = % purity x % germination/10,000

Values close to 1 indicate high quality, while numbers close to zero indicate poor quality seed.

For example; Rhodes grass seed with a purity of 96% and germination of 50% has a PLS of 0.48, which means that 48% of the seed lot by weight contains seeds that can germinate. Typical PLS values for sub-tropical grasses are 0.4 - 0.6, but values as low as 0.07 have been measured. In a NSW study the average PLS for Katambora Rhodes grass was 0.52 from 18 tests, Premier digit grass was 0.40 from 52 tests and Bambatsi panic 0.57 from 39 tests (G. Lodge and L. McCormick 2010)

Seed storage and its effect on germination

Panic grass seed has post-harvest seed dormancy. The most effective method to break this dormancy is time, whereby seed is stored until the dormancy breaks down naturally. Priming agents (e.g. gibberellic acid, ethylene, karrikinolide) are effective at breaking seed dormancy under laboratory conditions, but have had limited benefit in field trials to date.

Wholesale seed merchants are unlikely to store panic grass seed for more than 12 months after harvest, without substantially increasing seed price to cover additional storage costs. Therefore, an option for producers who want to sow panic grass seed with maximum levels of germination is to purchase seed 12 months in advance of planned sowing.

This raises the question of “What storage conditions are best to maintain viability of both panic grass and Rhodes grass seed”? To answer this, an experiment was conducted using seed of green panic and Katambora Rhodes grass under different storage conditions. Seed was harvested from Medina Research Station in early March 2007 and graded to ~100% viability using a gravity-fed aspirator and then confirmed with an x-ray machine that checks for seed-fill. Seeds were then stored for 12 months under three conditions:

(i) A temperature controlled seed storage facility with a relatively constant temperature of 18^{o}C;

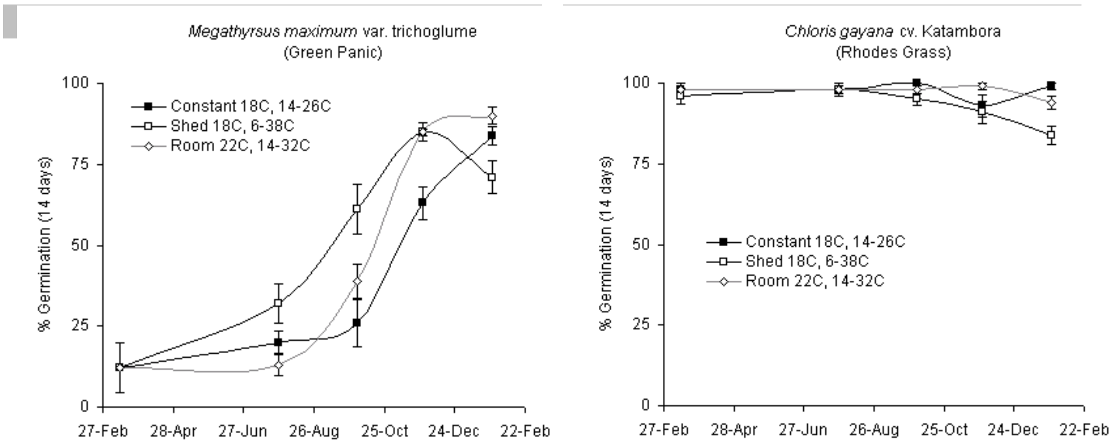
(ii) A laboratory at room temperature (average of 22^{o}C and range of 14-32^{o}C); and

(iii) A garden shed (average of 18^{o}C and range of 6-38C)

Seed was germinated at regular intervals under optimal conditions for each species (alternating day/night temperatures of 26/13°C). Results are shown below.

Germination of green panic grass immediately after harvest was ~13%, in contrast to almost 100% for Katambora Rhodes grass. Germination of green panic increased over time under each storage condition, with maximum germination being reached by November, for seed stored in the shed and at room temperature, and by January for seed stored in the temperature controlled facility. Germination of both species stored in the shed declined somewhat after November, suggesting that high summer temperatures reduce viability. However, germination for Rhodes grass was still >80% after 12 months.

It should be noted that seed stored for more than 12 months may decline in quality and germination should be re-tested before sowing.



The effect of storage on germination for: (a) Green panic, showing loss of dormancy over time; and (b) Katambora Rhodes grass, which does not have post-harvest seed dormancy. Seeds were either stored in (i) a controlled temperature storage facility; (ii) a laboratory at room temperature; or (iii) a shed. The average temperature and temperature range are shown for each storage situation.

6. Pre-sowing weed control

It is essential to sow sub-tropical perennial grasses into a weed-free seed bed, as their seedlings are weak competitors with both established winter growing pasture plants and newly germinated summer growing weeds. There are several ways of achieving a weed-free seed bed, each of which needs to be balanced against the potential erosion risk, particularly immediately after seeding when the paddock is highly susceptible to wind erosion. The aim should be to minimise this risk, especially on exposed sites with loose sandy soils. Depending on seasonal conditions and soil type, there is a trade-off between the method and timing of weed control and the amount of decaying root biomass remaining to bind the soil post-seeding.

For erosion-prone sites, develop a weed control strategy that ensures complete weed control at seeding, but leaves some decaying root mass to help bind the soil and prevent erosion. Alternatively, sow a cereal crop in year 1 and then sow the sub-tropical grasses into the standing stubble in year 2.

6.1. Weed control strategies

The ideal paddock preparation is to reduce weed seed set a year ahead of sowing by grazing and spray-topping.

Six to eight weeks before the intended sowing date assess the paddock for pasture composition. Identify weeds which will be difficult to kill with a single knockdown herbicide, such as silver grass, erodium, subterranean clover, capeweed, wild radish, doublegee and Paterson's curse.

Three general weed control strategies, detailed in Table 2, are:

(a) a single knockdown herbicide around 2 weeks before sowing;

(b) a selective broadleaf spray around 6 weeks before sowing, followed by a knockdown herbicide 2 weeks before sowing; or

(c) a double knockdown strategy with applications of knockdown herbicide at around 6 weeks and 2 weeks before sowing.

Many producers are now using either a single knockdown together with a selective broadleaf herbicide or, a selective spray targeting ‘difficult to control weeds’ followed by a knockdown herbicide.

In each case, excess biomass should be removed by grazing pre- and/or post-spraying.

Existing pasture must be completely killed before sowing the perennial grasses. Because they are much larger in late winter they require higher rates of knockdown herbicide (with appropriate additives) than that needed to kill them earlier in the season.

Producers commonly add selective broadleaf herbicides to the knockdown along with ammonium sulphate and wetter to improve the spray efficacy, especially for difficult to control weeds (refer to the Case Studies).

Applying an early knockdown herbicide (about 6 weeks before sowing) means the effectiveness of the spray can be assessed and there is the option of applying a second spray to any areas with incomplete weed control. Applying the knockdown herbicide early can also result in a small amount of additional stored soil moisture. The change in stored soil moisture is relatively small as water loss from a bare soil or annual pastures over winter are similar.

Table 2. Weed control strategies for establishing sub-tropical perennial grasses

Strategy	When to use[1]	Description of strategy	Advantages	Disadvantages
(a) Single knockdown	Low density of weeds 6 weeks before sowing, which are difficult to kill when mature	Graze to reduce excess biomass Use a single knockdown herbicide (glyphosate + additives) at least 2 weeks before seeding[2].	Decaying annual pasture roots help bind the soil together during establishment More time to assess seasonal conditions and defer seeding if seasonal conditions are unfavourable Additional winter grazing compared with other strategies Lower cost than other strategies	Full weed control may not be achieved, resulting in increased competition for establishing grasses More difficult than double knockdown strategy for precise seed placement, as soils tend to be more cloddy Slightly less stored soil moisture for establishing seedlings
(b) Broadleaf selective spray followed by a knockdown	Moderate density of weeds 6 weeks before sowing, which are difficult to kill when mature	Use a broadleaf selective spray or the spray-graze[3] technique to target difficult to kill broadleaf weeds at least 6 weeks before seeding Graze to reduce excess biomass Apply a knockdown herbicide (glyphosate) over the paddock at least 2 weeks before seeding[2]	Decaying annual grass roots help bind the soil together during establishment Seeding can be deferred after selective spray if unfavourable seasonal conditions for sowing, while still maintaining grass cover Additional winter grazing compared with double knockdown strategy	Cost of additional herbicide application, compared with single knockdown More difficult than double knockdown strategy for precise seed placement, as soils tend to be more cloddy Slightly less stored soil moisture due to additional annual pasture growth than with the double knockdown strategy

(c) Double knockdown	Many weeds 6 weeks before sowing, which are difficult to kill when mature	Graze to reduce excess biomass Apply first knockdown herbicide ~6 weeks before seeding (glyphosate) If further weed control is needed, apply a second knockdown (glyphosate or SpraySeed) at least 2 weeks before seeding[2]	Excellent weed control normally achieved Lack of decaying root biomass reduces cloddiness, making precise seed placement easier More stored soil moisture for establishing seedlings from extended weed kill	Increased erosion risk due to less decaying root biomass binding soils together. Reduced winter grazing with early knockdown Cost of additional herbicide application, compared with single knockdown strategy More difficult to defer seeding to the following year if seasonal conditions are unfavourable

[1]Assess paddock 6 weeks prior to planned sowing date

[2]Apply knockdown herbicide at least two weeks before seeding to ensure plants are dead.

[3]Spray-grazing, using phenoxy-based herbicides followed by intense grazing, is effective at controlling broadleaf weeds like erodium, capeweed, Paterson's curse and wild radish, as the herbicide increases the palatability to stock and causes flat weeds to curl up, making them more accessible.

Poor control of annual weeds

Good control of annual weeds

This shows the importance of good annual weed control for successful establishment and strong early growth of sub-tropical grasses. The front plot was sprayed with glyphosate (2 L/ha) 6 weeks and 2 weeks prior to sowing (double knockdown), which achieved excellent weed control. However, the rear plot was only sprayed 1 week prior to sowing with glyphosate (2 L/ha) (a single knockdown), which was not sufficient to kill the existing pasture plants – these sub-tropical grasses subsequently died over summer. Photo taken at Geraldton on December 2, 2009, following sowing on August 25.

Effect of different weed control strategies

In order to test the effect of different pre-emergent herbicide strategies on establishment, an experiment was conducted on a deep, non-wetting sand at Gillingarra (150 km north of Perth). Three treatments were compared, consisting of:

(i) A single knockdown sprayed on August 15 (glyphosate @ 1L/ha);

(ii) A double knockdown sprayed on July 22 (glyphosate @ 2L/ha) and August 15 (glyphosate @ 1L/ha); and

(iii) 2-4,D ester @ 700 mL/ha for broadleaf control on July 22, followed by glyphosate @ 1L/ha on August 15

The Evergreen Northern Mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) was sown @ 4 kg/ha on August 27 in a randomised block design with three replicates. Plot areas were 150 m length x 40 m wide. Nine plastic tags were randomly placed in the bottom of seeding furrows in each plot 5 days after sowing to measure sand in-fill into furrows. Seedling counts and sand in-fill were measured 47 days after sowing. The results are shown in Table 3.

Table 3. Establishment density 47 days after sowing of perennial grasses (Evergreen mix) and sand in-fill into furrows following three different pre-emergent herbicide treatments on a loose, sandy soil at Gillingarra, WA.

	Pre-emergent herbicide treatment		
	1 knockdown	2 knockdowns	Broadleaf selective then knockdown
Plant density (plants/m^2)	13.6	27.2	21.0
l.s.d. (P = 0.05)	5.4		
Sand in-fill (mm)	6.4	12.0	8.8
l.s.d. (P = 0.05)	2.5		

The double knockdown herbicide treatment had the highest seedling establishment. Examination of the seedbed showed it to be less cloddy than the other herbicide treatments, which presumably resulted in more accurate seed placement. However, the selective broadleaf treatment still gave a high establishment density, which was much higher than the single knockdown treatment.

Sand in-fill into furrows was highest for the double knockdown treatment and was similar for the other two treatments. Had severe wind events occurred in the 4 weeks after sowing, it is likely that establishment in the double knockdown treatment would have been adversely affected.

This suggests a broadleaf selective herbicide, followed by a knockdown herbicide, is a good compromise between the requirement for a weed-free seedbed and reduced erosion potential.

Can cereals be used as a windbreak to reduce erosion risk during establishment?

One option for reducing the risks of seedling losses from wind erosion is to sow a companion cereal, either in a mixture with the sub-tropical perennial grasses or as separate rows.

In order to test the effect of sowing separate cereal rows, a trial was sown on a sandy soil at Geraldton on August 25, 2009. The Evergreen Northern mix (60% Gatton panic grass, 20% Katambora Rhodes grass, 20% signal grass) was sown @ 4 kg/ha. Oats or sorghum were sown every 6th row or 9th row in place of perennial grasses.

The overall conclusion was that the cereal barriers were too small to be effective in minimising wind erosion during the first six weeks while the perennial grass seedlings were establishing. In late spring – early summer the oats and sorghum were an effective barrier, but by this time the perennial grasses were established and the risk of erosion was low (see photo). Other work has shown that sowing oats in a mixture with the perennial grasses also has a direct adverse effect on their establishment, due to competition for moisture. Producers have reached similar conclusions using cereal rye as the windbreak.

More practical solutions for reducing the potential for wind erosion in susceptible paddocks are:

- Sowing a cereal crop the year before sowing sub-tropical grasses; and then sowing the perennial grasses into the standing stubble, which acts to protect the soil surface from erosive winds.

- Using a pre-emergent weed control strategy that leaves some decaying root residues during establishment.

Companion cereal trial near Geraldton, showing the Evergreen sub-tropical grass mix with sorghum (foreground) and oats (background) sown every 6th or 9th row. The usefulness of sorghum and oats in reducing the erosion risk during the critical establishment phase was limited by their slow early growth. Photo taken December 2, 2009, following sowing on July 25.

7. Sowing time

Sub-tropical grasses require soil temperatures above ~15-18°C for germination, so sowing time needs to be late enough for soil temperatures to be sufficiently warm.

The key is to strike a balance between rising soil temperatures in late winter and early spring versus the likelihood of follow-up rainfall after seeding. As a result, the ideal sowing time is a compromise between sowing early enough to enable sufficient root development prior to onset of the summer drought and late enough so that low soil temperatures do not limit germination and growth.

This leads to a *sowing window* for each location, which is a range of sowing dates that optimizes the chance of successful establishment. The sowing window is earlier in the year for warmer and lower rainfall areas than for cooler, higher rainfall areas.

As a guide, sowing windows for the main regions in WA suited to sub-tropical grasses are:

- Dongara-Kalbarri districts - early to late August
- Perth-Eneabba districts - mid-August to early September
- South coast - early September to early October

Sub-tropical grasses must be sown into moist soil or where there is a very high probability of rain in the next few days after sowing. Once sown, they can establish successfully with post-seeding rainfall from September to November of only 25-

50 mm, provided there is good stored moisture in the soil profile for the seedlings to access and allow the plants to establish before the onset of the summer drought.

If soil moisture conditions are not favourable for germination and there is little prospect of imminent rain during these sowing windows, sowing should be deferred until the following year.

Sowing window for sub-tropical grasses – an example

The ideal sowing time to maximise establishment of sub-tropical grasses in WA can be termed their *sowing window*. It is a compromise between sowing late enough so that soil temperatures do not limit germination and sowing early enough for sufficient root development prior to onset of the summer drought. The sowing window varies with location and is earlier for warmer and lower rainfall areas than for cooler, higher rainfall areas.

In order to determine the sowing window at Gillingarra (150 km north of Perth), a field trial was conducted using Gatton panic and Katambora Rhodes grass on a sandy duplex soil. The trial consisted of 10 weekly sowing dates from July 25 to September 26. The trial area was sprayed with glyphosate on July 18 and again one week prior to each sowing. This routine continued until the 6th sowing (August 29), after which all remaining unsown plots were sprayed.

The site received 130 mm rainfall from July to September and maximum soil temperature rose above 15°C in the week following July 25. Large differences in panic grass establishment were observed, while Rhodes grass establishment was reasonably consistent over a range of sowing dates.

Grasses emerging from the first two sowings had to compete with a high annual weed density, whereas good weed control was achieved when the second knockdown occurred on or after August 8.

These results suggest the sowing window at Gillingarra is from mid-August to early September. This optimises high establishment density, low weed burden, good persistence over summer and high autumn biomass (Figures 3, 4)

Establishment of Rhodes grass (R) and panic grass (P) showing annual weed residues from sowing on July 25, August 15 and September 12 in 2007 at Gillingarra, WA. Photo taken on November 1.

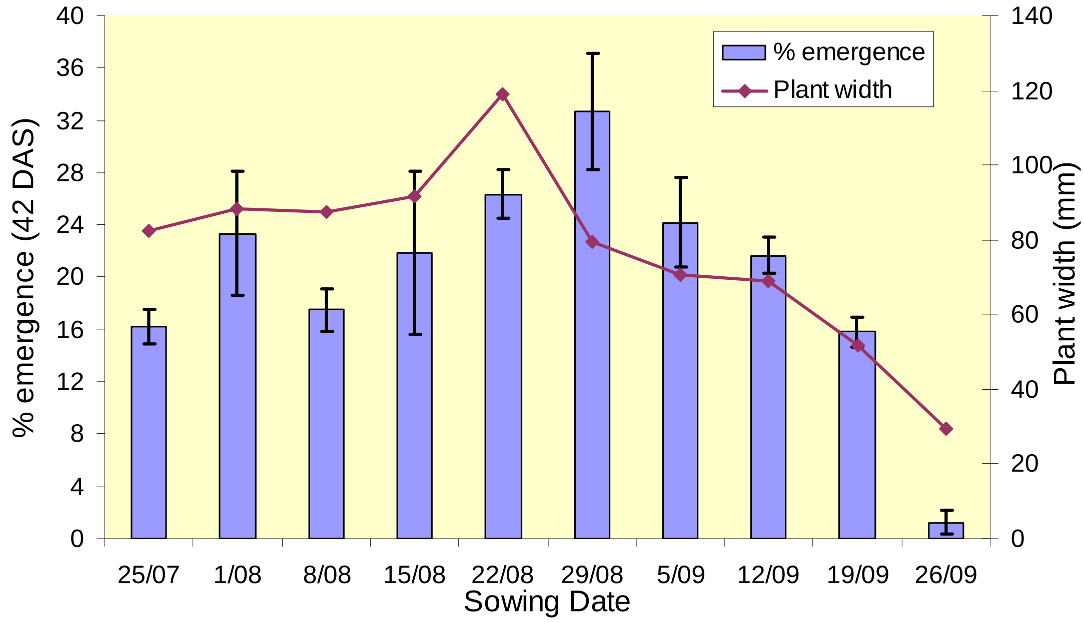


Figure 3. Panic grass seedling emergence (percent of germinable seeds sown) 42 days after sowing (DAS) and plant width (mm) on October 24 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors for % emergence.

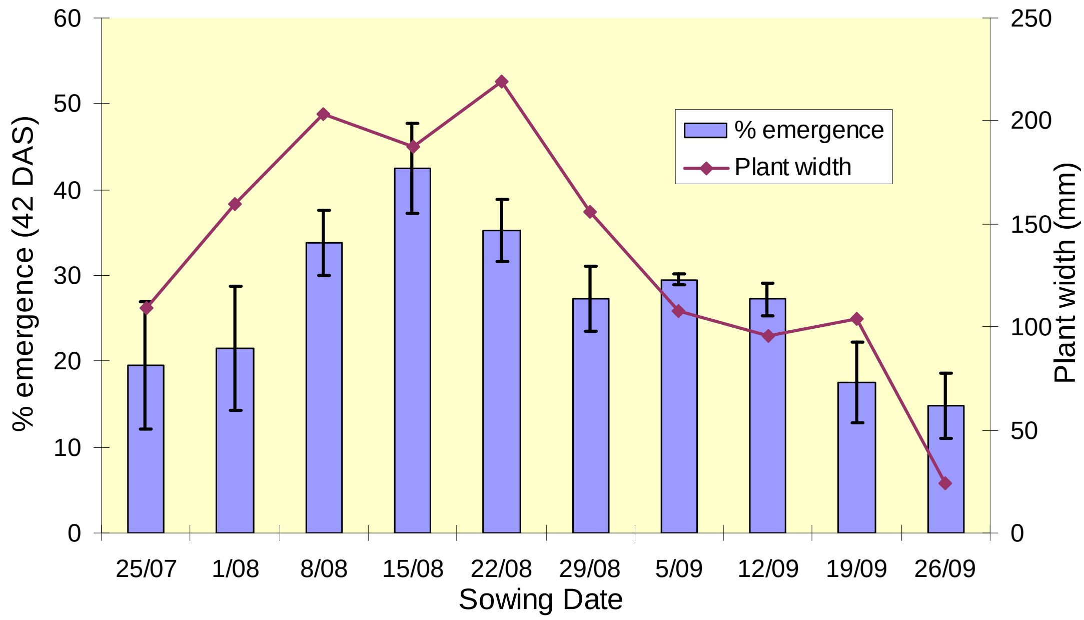


Figure 4. Rhodes grass seedling emergence (percent of germinable seeds sown) 42 days after sowing (DAS) and plant width (mm) on October 24 from ten weekly sowing dates in 2007 at Gillingarra, WA. Bars show standard errors for % emergence.

8. Machinery set-up

Sub-tropical grasses in WA are often sown on infertile, sandy soils that are non-wetting on the surface and have a low water holding capacity. For successful establishment the non-wetting surface soil needs to be scalped away and seeds need to be placed so that developing seedlings can make use of any soil moisture.

Successful establishment of sub-tropical grasses can be achieved with a range of seeding machinery and configurations (tynes, discs) provided the machine:

- forms stable furrows that scalp away non-wetting sand, remove weed seeds and harvests rainfall
- uses press wheels to provide good seed contact with moist soil
- has a wide row spacing (typically 50-60 cm)

For example, many producers are using converted culti-trash combines with widely spaced discs and press wheels to sow sub-tropical grasses – refer to Machinery case studies. Triple disc drills, conventional combines and air seeders that are widely used for sowing crops may need some modifications to give reliable establishment.

8.1. Furrow sowing

On sandy soils furrow sowing is important to scalp away the non-wetting sand, to remove weed seeds from near the perennial grass seedlings and to harvest rainfall. Furrows capture rainfall and increase seedling survival, particularly in dry springs. They act to turn relatively small rainfall events into useful soil moisture for growth and survival. For example, 3-5 mm of rain can effectively become 10-15 mm for seedlings in the bottom of a furrow.

Furrow depth can be varied according to seasonal conditions. The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting.

Furrow shape should be designed to minimise furrow collapse and sand in-fill, as this causes burial of the seed, which can markedly reduce seedling emergence. Wide furrows with sides that are not too steep are less prone to collapse and sand in-fill (Figure 5).

Seeding without furrows is risky, as it relies on favorable spring-summer rainfall, and is more likely to result in poor or patchy establishment, particularly in years with low spring and summer rainfall.

A number of producers have converted old culti-trash combines to form furrows (50-100 mm deep) that remove the non-wetting sand, enabling the perennial grasses to be sown into moisture in the bottom of the furrow (refer to Case Studies). To create furrows of a similar size, tynes may result in more loose soil than discs. The discs tend to peel the soil back, invert it and drop it in the inter-row, creating a well defined furrow.

To form large furrows some producers have developed modified tynes to get the required soil movement and furrow formation, but this can result in a large amount of loose soil on the soil surface. This is less of an issue with smaller furrows. Another option with tynes is to use a depth wheel which gives precise seeding depth control and compacts the side of the furrow.

8.2. Press wheels

Press wheels provide good seed contact with soil moisture and lead to more reliable germination. They should press soil around the seed in the furrow bottoms and minimise sand in-fill from the furrow sides. Many producers simply drop the seed into the bottom of the furrow and use press wheels to push the seed below the soil surface. This system positions the seed well for germination, but strong press wheel action is essential to give good seed-soil contact.

The contour of the press wheels should fit within the furrow shape to minimise sand in-fill. The base of the furrow should be wider than the press wheels. Rounded or flat-bottomed wheels tend to produce a better result than angular, narrow wheels, while ”W”-shaped press wheels are the least effective (Figure 6). It is also important to ensure that press wheels are correctly aligned with the furrows and that the arms allow consistent tracking along furrows.

8.3. Row spacing

Producer experience has shown a row spacing of 50-60 cm is generally ideal. This ensures a sufficiently wide inter-row so that any soil movement during sowing from one furrow does not cause sand in-fill into adjacent furrows. It also provides sufficient spacing for annual pastures to grow between the rows in the cooler months, thereby providing a good balance between perennial and annual pasture components.

A wider row spacing can be used in low rainfall areas to reduce competition for soil moisture. Row spacing can also be adjusted to change the balance between winter-spring feed from annual pastures in the inter-row versus potential out-of season production from the perennial grasses.

Close row spacing, such as that achieved with a conventional seeder, can result in greater competition for water, resulting in small, stunted plants and reduced growth following out-of-season rainfall.

8.4. Sowing different grass species in alternate rows to reduce competition

In mixtures of sub-tropical grass species, pasture composition can be dominated by the species with higher seedling vigour. For example, Rhodes grass has the highest seedling vigour of the commonly grown sub-tropical grasses and often out-competes other species when they are sown together, adversely affecting their establishment (Table 4).

To overcome this, some producers sow mixtures of Rhodes and panic grasses in alternate rows to ensure the pasture composition reflects the seed mix. Simple dividers can be used in the seed box to allow species to be sown in alternate rows (see photo).

Alternate row sowing - panic grass with Rhodes grass and signal grass in the alternate rows

Sowing panic and Rhodes grasses in alternate rows

In species mixtures, the species with the most vigorous seedlings tend to out-compete those with less vigorous ones during establishment. Rhodes grass has a more vigorous seedling than panic grass and consequently it often ends up dominating the pasture when they are sown together. One way to overcome this is to sow Rhodes grass and panic grass in alternate rows.

To demonstrate this principle a series of plots were sown near Geraldton on August 25, 2009. Gatton panic and Katambora Rhodes were sown at the same sowing rates as a mixture or in alternate rows (i.e. 0.75 kg/ha of Gatton panic and 2.0 kg/ha (coated) of Katambora Rhodes). Seedling densities were compared 47 days later.

Table 4 shows the panic grass seedling density was much higher when sown in alternate rows than when sown together with Rhodes grass as a mixture in the same row.

Table 4. Plant density of Gatton panic and Katambora Rhodes grass 47 days after being sown at Geraldton as a mixture or in alternate rows. Panic grass was sown at 0.75 kg/ha and Rhodes grass at 2.0 kg/ha (coated)

Sowing method	**Species**	**Plant density (plants/m^2)**
Mixed rows	Panic grass	2.7
	Rhodes grass	10.9
	Total	13.6
Alternate rows	Panic grass	9.7
	Rhodes grass	7.6
	Total	17.3
l.s.d. (P = 0.05)		3.5

To sow different species in alternate rows use dividers in the seed box

9. Sowing rate

Sub-tropical grass seed is not cheap, so it is important to carefully calibrate the seeder to ensure the correct amount of seed is being sown.

Sow 2-5 kg/ha of seed - depending on seed quality and whether the seed is coated. As a guide, for uncoated seed with 40% germination, 2 kg/ha is sufficient to give a good seedling density. Higher rates should be used for seed of lower germination. Higher rates are also required for coated seeds to sow the same number of seeds per area.

Kikuyu seed normally has high seed quality (> 80% germination) and sowing at 1 kg/ha is usually adequate. However, full groundcover will be achieved more rapidly by sowing at 2 kg/ha.

With light, fluffy seeds like Rhodes grass, it is often necessary to use a carrier if the seed is not coated. Use a low rate of fertiliser (e.g. 20-25 kg/ha) mixed with the seed for better flow through the machinery.

10. Sowing depth

Sub-tropical grass seeds are small and therefore, require precise, shallow seeding at a depth of 5-10 mm. For example there are ~3.3 million seeds/kg for uncoated Rhodes grass and ~1.2 million seeds/kg for panic grass, compared with ~150,000 seeds/kg for subterranean clover. Their small size means most sub-tropical grass seeds have insufficient energy reserves to emerge from depths much greater than 10 mm. **Seeds sown too deep will not emerge.**

Good seeding depth control can be achieved using a precision seeder designed for small seeds. However for producers without precision seeding equipment, the simplest method is to drop the seeds in the bottom of furrows and press them into the soil with press wheels to ensure good seed-soil contact. This is an effective method which has been widely used by producers – refer to Case studies.

Seeding depth should be checked to ensure that the majority of seeds are being placed 5-10 mm below the surface in the bottom of furrows. This should be done at normal operating speed. When correctly adjusted, a small proportion of sown seeds are usually visible on the soil surface. The bright colour of coated seeds makes checking seeding depth relatively easy.

Care must be taken to minimise furrow collapse and sand in-fill into furrows, as this can bury seeds beyond their ideal sowing depth and reduce their chances of emerging. In the first 2-3 weeks after sowing there is often some sand in-fill in the furrow which increases the 'effective seeding depth' (sowing depth plus sand in-fill).

Effect of sowing depth on emergence

Sub-tropical grasses have small seeds, but just how critical is sowing depth to their chances of successful establishment?

A field trial was conducted under irrigation on a sandy soil at South Perth to determine the optimum sowing depth for establishment of Gatton panic, Katambora Rhodes grass, signal grass, Whittet kikuyu and Splenda setaria. Two sowing dates were used (August 24 and September 28).

The highest establishment densities for all species occurred from a sowing depth of 5-10 mm, indicating this is the optimum depth for sowing (Figure 7).

Rhodes grass and panic grass are small-seeded species and proved very sensitive to sowing depth. No Rhodes grass seedlings emerged and less than 50% of panic grass seedlings emerged from 15 mm or deeper. The larger seed of signal grass meant it was able to emerge over the range of 5-40 mm. Kikuyu also had some emergence from 20-30 mm (Figure 7).

No seeds emerged from the surface-sown treatment. However, seeds in this treatment were placed on the soil surface without being pressed or rolled into the soil.

Field experience has shown that successful establishment can be achieved when seeds are placed in the bottom of furrows and pressed into the soil with press wheels. This method works, as the seeds effectively end up 5-10 mm below the soil surface – the ideal position for successful establishment.

Seedling establishment of five sub-tropical grasses was compared at six sowing depths (surface-sown, 5, 10, 15, 20, 25 and 30 mm) at South Perth

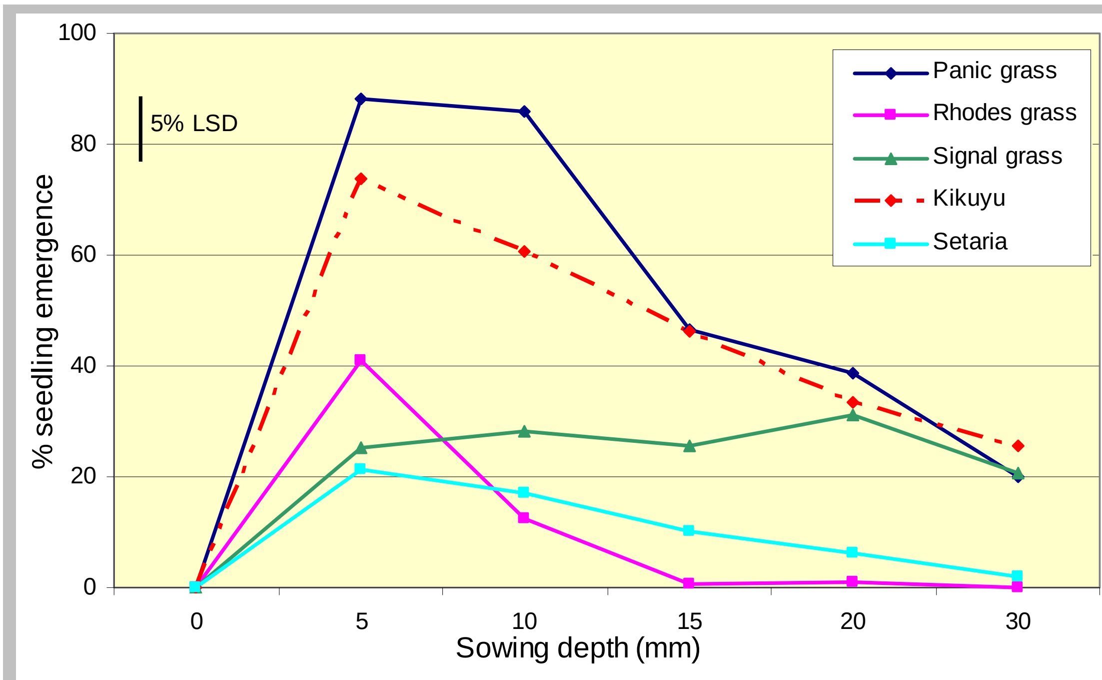


Figure 7. Seedling densities of five sub-tropical grasses at six sowing depths (surface sown, 5, 10, 15, 20, 25 and 30 mm) on a sandy soil at South Perth (mean of two sowing times). Note: surface-sown seed was not pressed or rolled in.

11. Sowing speed

Tractor speed can impact on establishment success. Driving too fast can cause excessive soil movement, reducing the accuracy of seed placement. Sand in-fill increases and furrows can collapse, resulting in a deeper effective seeding depth and poor establishment. Optimum speed varies with the type of machinery, but from experience speeds of 5-10 km/h generally give a good result.

Some machinery modifications can be made to enable sowing speed to be increased. For example, some producers have added rubber guards to prevent soil from one furrow being thrown into adjacent furrows.

To ensure the majority of seeds are being placed 5-10 mm below the surface sow at normal operating speed, stop and check the results. Continue to check seed placement regularly and make adjustments if needed.

Speed kills …!

A field trial was conducted at Gillingarra to investigate the effect of different sowing times on sub-tropical perennial grass establishment.

A Massey Ferguson combine seeder with scarifier points, soil rider depth control and press wheels was set up to sow the Evergreen Northern mix (60% Gatton Panic, 20% Katambora Rhodes grass, 20% Signal grass) at 5 kg/ha to a depth of 5-10 mm when driven at 5 km/h. Plant establishment following sowing at this speed was compared with that sown at 10 km/h and 15 km/h. Plots were 2.5 m x 48 m and treatments were replicated three times in a randomised block design. Seedling counts were conducted 42 days after sowing.

The results clearly show the effect of sowing speed. The seeder was set up to sow at 5 km/h and establishment from this sowing speed was highest. When sowing speed was increased to 15 km/h, the establishment density was less than 25% of that at 5 km/h (Table 5).

This shows:

- Seeding depth needs to be checked at the planned operating speed; and
- Speed needs to be reduced if too much soil movement is causing seeds to be sown too deep.

Table 5. Establishment density of sub-tropical grass seedlings 42 days after sowing at three different speeds at Gillingarra

Sowing speed	**Seedling density (plants/m^2)**
5 km/h	42.9
10 km/h	23.4
15 km/h	10.8
l.s.d. (*P* = 0.05)	18.4

12. Post-seeding checks

There are a number of post-seeding checks to ensure successful establishment of perennial grasses.

12.1. Identify emerging grass seedlings

In order to determine whether emerging grasses are those that were sown, the different species can be identified at the seedling stage using DAFWA Bulletin 4775, 'Identifying sub-tropical grass seedlings'.

12.2. Insect control

There are a number of insects which can damage perennial grass seedlings including cutworm and webworm caterpillars, redlegged earth mite, Rutherglen bugs and aphids. Apply a residual insecticide at sowing or with the last knockdown herbicide to control insects.

Regularly monitor the paddock (at least once per week) for insect damage, especially over the first 8 - 10 weeks when grass seedlings are emerging and are most vulnerable to insect attack.

12.3. Summer growing weeds

Summer growing weeds compete strongly with sub-tropical grass seedlings for soil moisture. Control of these weeds will optimise establishment of sown sub-tropical grasses. Broadleaf weeds such as Afghan and paddy melons, wireweed and fleabane can be readily controlled with a number of broadleaf herbicides, provided they are actively growing.

Couch grass is a highly competitive summer-active perennial grass that will out-compete the more desirable sown perennial grass seedlings during the establishment phase. Care needs to be taken with couch grass, as it often recovers from a knockdown herbicide applied in winter. However, once established, the sown sub-tropical grasses are more productive and compete strongly with couch grass.

Special consideration needs to be given to erosion-prone soils when perennial grass seedling density is low (< 5 plants/m^2). Summer weeds should be retained in such situations to reduce the erosion risk.

12.4. Kangaroos and rabbits

Kangaroos and rabbits can damage or kill young sub-tropical grass plants, so good control is essential. Unrestricted grazing can up-root establishing plants and increase stress over summer, which may reduce their persistence. In particular kangaroos will travel considerable distances to graze sub-tropical grasses, when little other green feed options are available.

Couch grass is very competitive with sub-tropical grass seedlings over the first summer

13. Grazing management

Careful grazing management over the first summer is critical to ensure a strong perennial stand in subsequent years. Sub-tropical grass seedlings have a weak primary root system and as a result are susceptible to up-rooting and grazing damage over the first summer. Uncontrolled grazing during this time will also deplete carbohydrate reserves which can result in plant death.

The timing of the first grazing will vary depending on seasonal conditions and how well the plants have grown. If late spring or summer rainfall has produced vigorous growth, the perennial grasses can be lightly grazed from early January. However, if very little or no summer rainfall has fallen, the first grazing will need to be deferred until after the break of season.

The first grazing should always be deferred until the perennial grasses are well established and well anchored.

Before the first grazing assess whether plants are firmly anchored by testing how easily they can be pulled out by hand. What appear to be relatively large plants can have a surprisingly small root system and in that case premature grazing would have a significant negative impact on the stand density with plants being uprooted and killed. Rhodes grass has long runners (stolons) that do not root down in dry soils and is, therefore, particularly susceptible to grazing damage in the first summer (test by pulling on the stolons).

If the plants are well anchored then a light grazing will encourage tillering. Monitor the perennial grasses while stock are in the paddock to ensure they are not being up-rooted or over-grazed.

On-going grazing management will vary with the perennial grass, grazing animal and time of year. Most perennial grass paddocks can be largely set-stocked by cattle during the growing season, but they will require some form of rotational grazing outside of the annual growing season. Kikuyu is highly grazing tolerant and can be set stocked for long periods by sheep or cattle, but leading producers are now rotationally grazing cattle on kikuyu pasture, with resulting large increases in productivity.

14. What is successful perennial grass establishment?

Successful establishment in the autumn after sowing, following a dry summer with minimal rainfall

Successful establishment is not simply the number of perennial grass seedlings that emerge in spring, but relates to the density of plants that persist through to the following autumn. Successful establishment equates to uniform plant density across the paddock with continuous lines of perennial grasses in each seeding row.

A desirable benchmark for sub-tropical grass establishment is a density of 8-10 plants/m^2 surviving though to the first autumn after sowing. For a row spacing of 50-60 cm, this equates to 4-5 plants per metre in each seeding row.

Bunch grasses, such as panic grass, setaria, digit grass and Bambatsi panic, do not spread by runners and rarely recruit new seedlings from seed. In general, the plant density of these species will not increase over time, although crowns of individual plants will get larger. Therefore, the medium to long-term plant density of the bunch grasses is largely determined by those plants that persist through to the end of the first autumn after sowing.

The creeping grasses, such as Rhodes grass, kikuyu and signal grass, are more forgiving of poor establishment density, as they will spread and fill in gaps over time, given appropriate management.

Small, weak plants in late November - early December are unlikely to persist over summer, unless there is good summer rainfall. On the other hand, plants which are strong and well established in late spring – early summer have a high likelihood of persisting over summer, even if faced with adverse seasonal conditions with little or no rain for the next 6-7 months.

Results from field trials suggest that 30-65% of seedlings in the October following sowing will not persist over the first summer (Figure 8). Obviously the attrition rate will vary with seasonal conditions, plant size in late spring-early summer and seedling density. If seedling density is excessive, the plants will compete strongly for moisture and the weaker plants will not survive.

Good establishment of panic grass in early December

To determine the success or otherwise of a newly sown perennial grass paddock, it is useful to assess the paddock at two scales:

(i) At the paddock scale look for uniformly good establishment across the paddock, consisting of continuous or almost continuous lines of perennial grasses in each seeding row. In other words, not patchy establishment with good areas, poor areas and other areas with few or no perennial plants.

(ii) In the immediate area around where you are standing (i.e. within 5 to 10 m), assess the plant density and composition (if a mixture was sown). Plant density can be determined by counting the number of plants per metre of seeding row. Pasture composition can be determined by estimating the relative number of each species, which should approximate the proportion of each species in the seed mix (i.e. relative number of viable seeds per kilogram).

Small stressed plants at the start of summer (early December) may not persist over summer unless there are favourable seasonal conditions

15. Trouble shooting

If establishment is sub-optimal or the resulting composition is not as planned, it is useful to identify the contributing factors, so they can be rectified for future sowings. Table 6 lists potential causes of poor establishment, following sowing of a mixture of panic grass and Rhodes grass.

Table 6. Possible causes of sub-optimal establishment from sowing a mixture of panic grass and Rhodes grass (these principles apply to other mixes)

Paddock observations	**Possible cause(s)**
Patchy or poor establishment	• Poor depth control or seed sown too deep • Sowing speed too high, resulting in poor depth control, furrow collapse and/or sand in-fill in furrows • Poor seed quality (either low germination and/or high proportion of dormant seed) • Poor or incomplete weed control (will generally be obvious) • Insect damage to newly emerging perennial seedlings
Low perennial density and mainly Rhodes grass	• Poor seed quality of panic grass (low germination or high proportion of dormant seed) • Poor depth control or seed sown too deep, while some Rhodes grass seeds on or near surface (possibly due to seed bounce) have germinated.
Good panic grass density, but little or no Rhodes grass	• Poor seed quality of Rhodes grass • Sowing depth too deep or sand in-fill has buried seed beyond 10 mm, resulting in little emergence of Rhodes grass
Low perennial density in the autumn after sowing, in spite of good perennial density in spring (i.e. poor persistence over summer).	• Plants being up-rooted from grazing before being securely anchored to the soil • Over-grazing by stock, rabbits or kangaroos, resulting in depletion of

	carbohydrate reserves and plant death. Apart from over-grazing or premature grazing, the most likely cause is a high proportion of small, weak perennial plants in late spring – early summer, followed by dry summer conditions causing death of the perennial grasses from moisture stress. Possible causes of small perennial grass plants in late spring – early summer include: • Late sowing, resulting in limited growth of the grasses by the start of summer • Seedling density in spring being too high (>30 plants/m^2), resulting in strong competition between plants • Competition from summer weeds (e.g. wireweed, Afghan and paddy melons) • Competition from couch grass that has recovered from knockdown spray(s) acting to suppress the seedling growth of sown perennial grasses • Incomplete kill of annual pasture or late germinating annual grasses (e.g. annual ryegrass) out-competing perennial seedlings

What is the optimum density of panic grass?

Two key questions relating to sub-tropical perennial grass pastures are:

(a) What is the ideal perennial grass density in a mature stand to maximise productivity? and

(b) What seedling density is needed to achieve this density in a mature stand?

These questions are particularly important for the bunch grasses,(panic grass, digit grass and setaria), as their medium to long-term density largely depends on the density of plants surviving through to the autumn after sowing.

To answer these questions, a replicated field trial was established at Badgingarra in August 2007 using Gatton panic seedlings transplanted at six different densities (0.5, 1, 2, 4, 8 and 16 plants/m^2). Biomass, pasture composition and persistence were measured at regular intervals.

Arrangement of the panic grass density trial at Badgingarra. Plots were sown at a density of 0.5, 1, 2, 4, 8 and 16 plants/m^2. Photo taken on June 11, 2009, following establishment in August 2007.

Figure 9 shows the total biomass of panic grass, annual legumes, grasses and herbs in the third season after sowing. At the lower densities the panic grass plants grew larger and remained greener over the summer period than plants in the higher density plots (Figure 10).

The results suggest the optimum plant density in the third spring-summer period in this environment is ~4 plants/m^2. However, this trial used glasshouse-raised transplanted seedlings, which gave them an advantage over the first summer compared with field-grown plants from seed. A more realistic density to aim for is 4-8 healthy plants/m^2, as this gives high biomass production from the grasses in the warmer months, after the annual species have senesced. It also provides sufficient space between plant crowns to allow production from annual legumes and grasses in the winter months, when the panic grasses are largely dormant or only grow slowly.

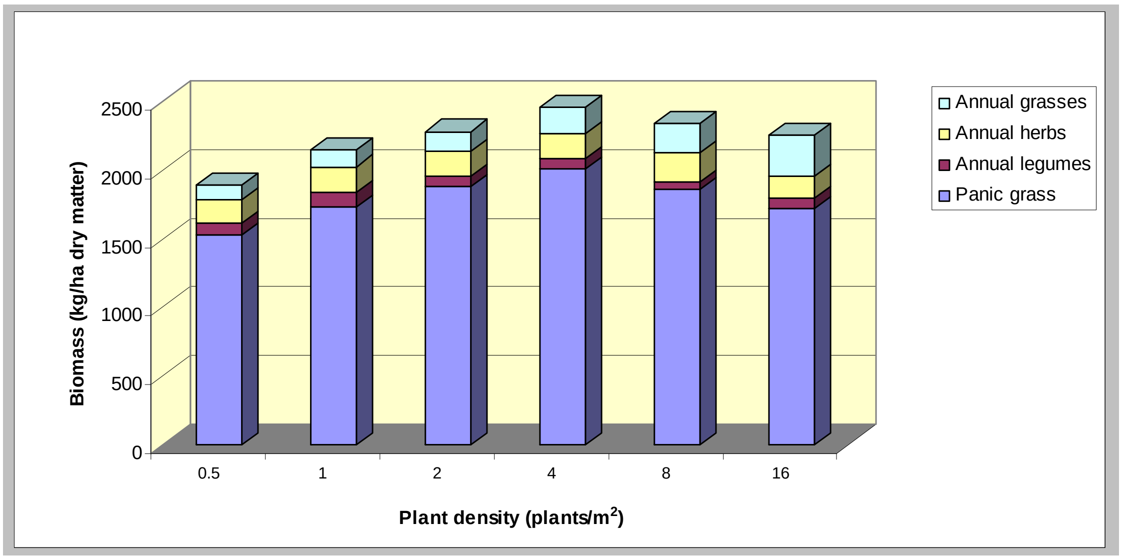


Figure 9. Biomass (total of four cuts) of panic grass, annual legumes, grasses and herbs in the third season (June 2009 to May 2010) after sowing at densities of 0.5, 1, 2, 4, 8 and 16 plants/m2 at Badgingarra

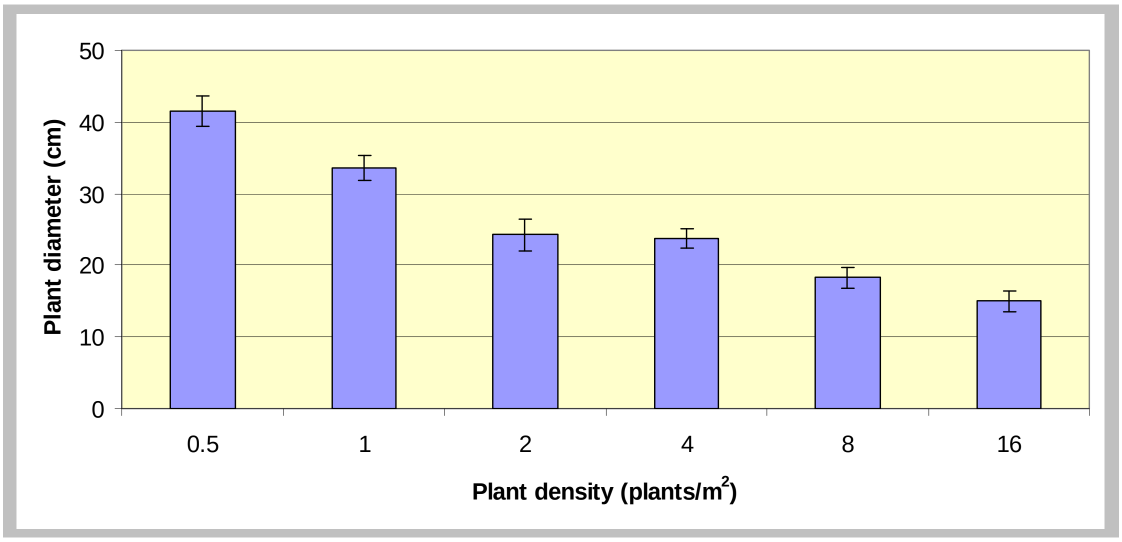


Figure 10. Diameter of Gatton panic grasses in December 2010 at densities of 0.5, 1, 2, 4, 8 and 16 plants/m2 at Badgingarra

16. Introducing companion annual legumes

A productive perennial grass pasture requires a companion legume to provide nitrogen to drive productivity of the system and improve feed quality during the growing season. On the sandy soils where sub-tropical grasses are often grown the best annual legume options are blue lupins, hard-seeded French serradella and yellow serradella. On gravelly soils and sandy duplex soils subterranean clover may be a suitable companion annual legume.

Many of the paddocks where sub-tropical grasses are established have little or no legume content in the pasture, so an annual legume has to be introduced either before or after the perennial grasses.

Four possible options are described below for establishing a hard-seeded annual legume, such as yellow serradella or hard-seeded French serradella (the soft-seeded French serradella cultivar, Cadiz, will not persist), which will regenerate each year and co-exist with the sub-tropical grasses.

(i) Establish the annual legume in year one and then sow the perennial grasses in year two.

This is the preferred method as the legumes can be managed as a seed crop in the first year to maximise seed-set. It is important to control insect pests, such as RLEM, aphids and particularly native budworm, which attacks green pods and can greatly reduce the seed yield. In year two heavily graze the re-generating annual legume pasture and then brown manure in winter, prior to establishing the sub-tropical grasses. The annual legumes will re-generate from hard seeds in the seed bank in the subsequent year.

(ii) Summer sowing

'Summer sowing' is an innovative method of establishing annual legumes by sowing seed or pod in an unscarified, dormant form. This has the advantage of reducing the need for further seed processing to enhance germination and in the case of serradella by avoiding the need for dehulling.

The concept is that pods of hard-seeded serradella are sown in late summer (February – March), which still allows time for a high proportion of hard seeds to soften before the break of season.

Most success to date has been achieved with hard-seeded French serradella (cultivars Margurita and Erica). These cultivars have a hard-seed breakdown pattern suitable for summer sowing, as about 50-60% of the seed softens each year. Commercial varieties of yellow serradella have a slower rate of hard-seed breakdown as only 20-30% of the seed softens each year and have a protracted germination pattern which is less suited to summer sowing (Figure 11). On the other hand, cv. Cadiz French serradella is 100% soft-seeded, so will not persist over successive seasons.

Summer sowing of hard-seeded French serradella cv. Margurita into established perennial grasses at Badgingarra (September 2010)

Preliminary results from field trials show that drilling pod is more efficient than broadcasting pod and using animals to trample it into the soil. It is preferable to achieve partial or full burial of serradella pods, as those sitting on the soil surface may not soften. High rates of serradella pod (20-40 kg/ha) may be necessary to achieve a satisfactory legume density and seed-bank. Growing and harvesting a serradella crop on-farm will reduce the cost of pod and make this method more economically viable.

(iii) Conventional sowing after the break of the season

In this method annual legume seeds are drilled into an established perennial grass pasture after the break of the season. Two to three weeks after the break of the season the paddock should be sprayed with a moderate rate of SpraySeed to reduce competition by annual pasture seedlings and to suppress the perennial grasses. Any well established broadleaf weeds from early germinating rains need to be controlled prior to sowing, as otherwise they will compete strongly with the annual legume seedlings.

Annual legume seed is then drilled into the perennial grass pasture, preferably with a disc drill to minimise damage to the perennial grasses. This method works best in years with an early break to the season, allowing the annual legumes to establish before cool winter temperatures. With later sowing, slow annual legume growth over winter risks seedlings being out-competed in spring by the perennial grasses.

(iv) Twin sowing

This method refers to sowing pods of hard-seeded serradella in spring at the same time as the perennial grass seed either in the same furrow or with pods broadcast in the inter-rows between perennial grasses. Field trial results to date suggest this method is unreliable for perennial grass-based pastures.

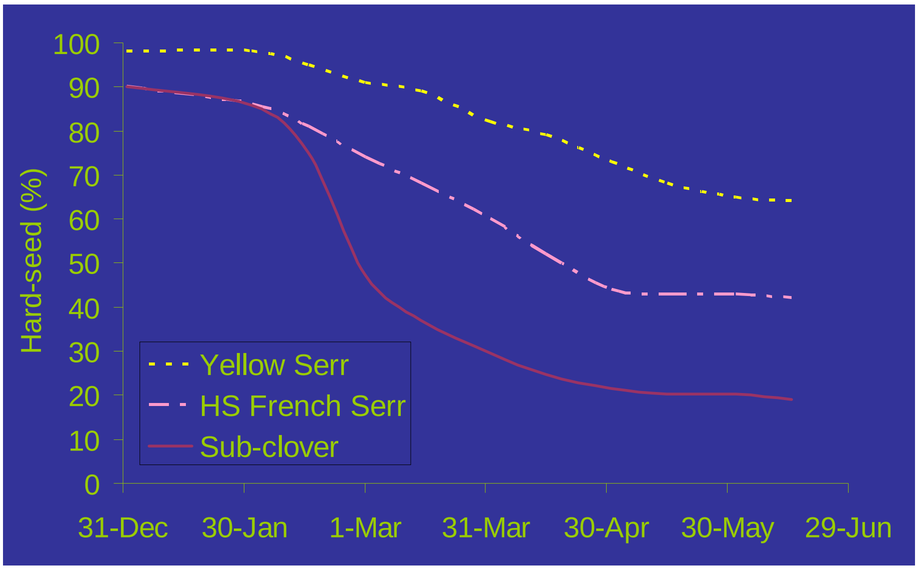


Figure 11. Hard-seed breakdown patterns of yellow serradella, hard-seeded French serradella and subterranean clover (B. Nutt unpublished data).

17. Machinery and seeding case studies

17.1. Modifying a culti-trash combine

Dan Rieusset from 'Koodiewoodie' in Dandaragan first sowed perennial grasses on his own property in 2005 and has been contract seeding since 2006. In 2009 his modified combine sowed 300 ha of sub-tropical grasses from a combination of contract seeding and hiring of the machine.

Dan modified his International A 6-2 24-run culti-trash combine following the lead of Grant Bain from Walkaway. He has made a number of modifications to the seeder and now consistently achieves excellent results.

"In terms of speed, I normally go about 5 km/h, but can go a bit faster in better and wetter sands."

"Initially seed and fertiliser were mixed together and sown through the fertiliser box, using around 20 kg/ha of super and 4 kg/ha of seed. A new method, using herbicide containers with funnels, along with changing the gearing and moving the slide in the seed box, makes seeding much quicker and easier."

"If I was to change anything, I would use discs that are more concave, rather than heating up and bending the journal (disc) carrying arms - to give a more consistent result."

Used herbicide containers inserted into the seed box allow low seeding rates

Machinery set-up:

International A 6-2 24 run culti-trash combine
Row spacing: 21” (52 cm)
Modifications to seeder: • International 511 culti-trash undercarriage • All back row discs and 2 out of 3 front row discs removed • New discs fitted • Heated up and bent journal (disc) carrying arms to improve the disc angle (for better furrow formation) • Welded a 50 x 50 mm RHS beam to rear journal (disc) carrying arms (to mount press wheels on) • Attached single K-Hart press wheels units • Fabricated new seed tube brackets and attached these to the RHS beam • Changed to the largest cog (18 teeth) on the gearbox (to reduce seeding rate) • Moved the slide at the bottom of the seed box to the finest setting (to further reduce seeding rate) • Inserted old herbicide containers and PVC pipe funnels over the 8 working seed outlets in the seed box (to enable seeding with only small amounts of seed)

17.2. Success using tynes

Producers: Peter Summers
Property: ‘Green Grove’
Location: Dongara
Total area: 4,000 ha (over 2 farms)
Arable area: 3,200 ha
Crop area: 2009: 80 ha (wheat)
Livestock enterprise: Self-replacing ‘multi-purpose’ Merino flock, selling wethers as lambs or 15-month old shippers
Livestock nos. Sheep: 4150 ewes, 500 wethers, 1500 ewe hoggets, 150 rams
Perennial pasture: 260 ha sub-tropical grasses
Annual rainfall: 420 mm

Peter Summers first sowed sub-tropical grasses in 2008 and says their benefits to his farming enterprise are erosion control and provision of more feed throughout the year. Peter has successfully established 260 ha of sub-tropical grasses over the last two years using a modified Massey Ferguson combine with two sets of points in-line, followed by press wheels. When asked if he would change anything on the seeder, Peter said “Adding more down pressure on press wheels - probably by adding extra weight.”

“The keys to successful establishment are weed control and furrow sowing. Avoid poor weed and rabbit control. In particular rabbit control needs to be achieved during the summer - autumn prior to sowing.”

“Successful sowing is good germination with almost continuous rows, with very few gaps.”

Peter is thinking of setting up another machine to use with the existing machine via a ‘twin-pull’. He will try a culti-trash conversion using the under-carriage from a MF500 combine on 28” spacing. If this doesn’t work, he will duplicate the current machine.

Peter has also set up a unique device for mixing seed and fertiliser. A small hopper has been located above the elevator on his 5:1 bin. This delivers pasture seed into the elevator while the fertiliser is passing through it. It has been calibrated so that the same amount of seed and fertiliser is mixed each time. The fertiliser box of the combine can be filled with 500 kg of the seed - fertiliser mix in approximately 2 minutes.




Machinery set-up:

24-run Massey Ferguson 56-tyne combine with floats from a MF 500 combine with: • 2 sets of points in-line • First point is a standard 5” point (to loosen the ground) • Second point is a modified 6” point with deflector plates (to remove non-wetting sand and create a furrow)
Row spacing: 28” (70 cm)
Modifications to seeder: • Moving the second tyne to be in-line with the first tyne (required a new bracket) • Deflector plates welded to points on second tynes (these measure 75 mm wide x 100 mm high, with a gap between them of ~ 25 mm) • Press wheels attached to the rear (unused) tyne mounts of the float (Primary Sales 100 mm V semi-pneumatic) • Seed tube directed in font of press wheel • Unused seed tubes blocked off with plastic covers in seed box

17.3. Persistence pays off!

Producers: Ken Hodby
Property: 'Rothesay'
Location: West Badgingarra
Total area: 2,800 ha
Arable area: 2,000 ha
Crop area: 2009: 60-80 ha (oats)
Livestock enterprise: Self replacing Angus cow herd, selling calves at weaning Merino ewe flock (bought in) joined to terminal Poll Dorset and Suffolk rams
Livestock nos. Cattle: 400 Angus breeding cows with calves, 60 yearling heifers Sheep: 1200 merino ewes with lambs
Perennial pasture: 260 ha sub-tropical grasses; plus some patches of couch (including giant couch), 10 - 15 ha of 20-year old overgrown tagasaste
Annual rainfall: 600 mm

Persistence has paid off for Ken Hodby, who successfully established 260 ha of sub-tropical perennial grasses in 2008 and 2009 after his first attempts at establishing the perennial grasses gave mixed results.

"I decided to get serious and converted a machine specifically for the job. I followed the best practice 'recipe' and attended an Evergreen 'Establishment Workshop' at Dandaragan in 2008."

“The keys for successful establishment are to sow into moisture and follow the ‘Establishment must-dos’. Good weed control is also essential.”

Ken gauges sowing success of sub-tropical grasses as a continuous row of plants in each sowing row. Commenting on the 2009 sowing, he said “The plant density is excellent and there was good dry matter production from the March rains.”

However Ken has been on a learning curve. “In 1995 I sowed 16 ha of Rhodes grass using a standard combine, but most of the seed was sown too deep. The result was 1 plant every 2-3 square metres and the bulls subsequently destroyed it.”

“I had a second go in 2002 with 24 ha. The paddock was ploughed up, seed dropped on top and then rolled in with a steel roller. It worked quite well, but a fire then burnt out lots of fences on the farm and the stand was grazed out over summer. In 2006 I tried the same technique as 1995, but the results were still poor.”

“The risk of erosion on high sandy ridges exposed to wind from all directions is the weakness in the system. In 2009, I sowed cereal rye down one row of the combine to try to create a mini-windbreak every 5 m across the paddock to reduce the risk of erosion. I will not do this again in future years. If the sub-tropical grasses germinate well, there is no need for the cereal rye, but if the spring is poor and the grasses fail, it is highly unlikely the cereal rye will do well enough to reduce the risk of erosion.”

Ken uses a modified Chamberlain Mk3 culti-trash 24 run combine with 21” (52 cm) row spacing after the front discs were removed and 2 out of 3 rear discs removed. The seeding tubes were extended to drop seed in the furrow just behind the disc and walking beam press wheel assemblies were added. The remaining discs were replaced, as more curvature leads to better furrows. The unused down tubes in the seed box were blocked off using flat tin.

“The ideal operating speed depends on the type of soil, so speed is adjusted by looking at soil throw behind the machine. If too much, slow down, if not enough, speed up.” Ken sows on the contour as some of his country is quite steep.

Asked if there was anything he would change on his seeder Ken replied, “Possibly add the capacity to spray a soil wetting agent in the bottom of the furrow before the press wheel or spray a slighter wider band (30 cm) so that the loose sand that falls back in to the furrow is also covered in wetting agent.”

Prior to sowing in 2010, the paddock had a large mob of cattle placed in it during April - May 2010 and these were fed supplementary hay. This allowed other pastures to get away, but also improved weed control on this paddock (particularly broad-leaf weeds).

Details of seeding program:

Year	2008	2009
Area sown	100 ha	160 ha
Paddock history	Long-term poor annual pasture	
Paddock preparation	Two sprays - July 8 and August 7 2 L/ha Glyphosate 40 ml/ha Hammer Li-700 Sulphate of Ammonia	Mostly single knockdown (August 11 - 17) 2 L/ha Glyphosate 40 ml/ha Hammer Li-700 Sulphate of Ammonia Insecticide *10% of the area had a second spray with the same mix as above on Aug 25 – these were thicker pasture areas and bits missed by the first spray*
Date of seeding	August 19 – 27	September 2 – 11
Seed mix	Evergreen Northern mix (60% Gatton panic, 20% Rhodes grass, 20% signal grass), which was mostly coated, except for signal grass. Used 1 bag of fertiliser for each bag of seed as a carrier, which was mixed in the fertiliser box of the combine.	
Seeding rate (kg/ha)	4	2 - 4 (should have been 4 but some calibration issues)
Soil type	Grey non-wetting sand (pH 5.5) over yellow sand, with limestone outcrops	
Soil moisture at seeding	Surface soil dry, but moist underneath	Excellent
Insecticide	No	Applied with knockdown

17.4. Sub-tropical grasses complement tagasaste

Producers: Bob and Anne Wilson
Property: 'Tagasaste Farm'
Location: East of Lancelin
Total area: 2,000 ha
Arable area: 1,800 ha
Crop area: 2009: Nil
Livestock enterprise: Self-replacing cross-bred cow herd Agistment cattle brought in during the growing season – numbers vary according to the season
Livestock nos. Cattle: 400 breeding cows (with calves at foot), 280 yearling heifers, 500 - 1000 agistment cattle (adjusted seasonally)
Perennial pasture: 367 ha sub-tropical grasses, 1,000 ha of tagasaste
Annual rainfall: 600 mm

A field walk inspecting a recent sowing of sub-tropical grasses with alleys of tagasaste at Bob Wilson's property near Lancelin

Cattle producer Bob Wilson is well known for promoting the benefits of tagasaste, but in recent years he has established a considerable area of sub-tropical grasses.

Bob sees the benefits of sub-tropical grasses to his farming enterprise as:

- using out-of-season rain to produce green feed

- better feed around the break of season and in late spring and early summer
- increased groundcover, reducing the risks of erosion
- evening out the annual feed supply – a bit less in winter, but more at other times

Bob used a Bettinson triple disc drill in 2003 and 2005, but in 2007 and 2009 he changed to a converted International 511 culti-trash combine.

“The Bettinson drill is not ideal for sowing perennials, because it doesn’t create decent furrows, but it did produce good results in 2003 and 2005. That said, in both years there were favourable springs. The Inter 511 drill probably gives a more reliable establishment of panic, whereas the Bettinson drill was pretty good with Rhodes grass.”




“I have also changed the seed mix slightly, with less Rhodes grass and more panic grass. The key to successful establishment is to follow the Establishment must dos,” said Bob.

“One knockdown spray has always worked well, except in areas of the paddock where erodium is present. This is particularly hard to kill, so I will move to a 2-spray program with a broadleaf spray first, followed by a glyphosate-based knockdown.”

“I check for insects every week or so after emergence, enough to notice insects moving in from adjoining paddocks.”

"The higher the plant density the better and in terms of the species mix – the more panic the better." Bob was happy with the 2009 sowing, as there was a good plant density over the whole paddock, particularly of panic grass.

The converted International 511 Culti-trash combine has a 14" (35 cm) row spacing. The back row of discs was removed and every second disc on the front row was removed. The remaining discs were swapped for heavier, scalloped discs and press wheels were added.

Furrow depth is ~ 50 mm and the sowing speed 5 - 7 km/h. However Bob said, "In areas with erodium present the machine does not dig in enough to create decent furrows - particularly on slightly harder soil types. I should probably adjust the sowing depth on the run."

"I mixed the seed with carrier fertiliser in a cement mixer in 2007, which was lots of hard work. In 2009 I mixed seed with carrier fertiliser in the fertiliser box of the combine – much easier when only using 25 kg/ha of plain super."

Details of seeding program:

Year	2003	2005	2007	2009
Area sown	62 ha	80 ha	95 ha	130 ha (22 ha to a mixture of Tagasaste and sub-tropical grasses)
Paddock history	Long-term poor annual pasture			
Weeds targeted	Capeweed, brome grass, blue lupins, erodium, silver grass (minor)			
Paddock preparation	2009: Grazed down hard until the end of July, then rested for 1 to 2 weeks before spraying on 17th August with 1 L/ha Roundup PowerMax + 150 ml/ha Sonic insecticide			
	Similar to 2009	Similar to 2009 with Hammer occasionally added to improve weed kill		(See above)
Date of seeding	First week of Sept.	Sept. 6 - 26.	Aug. 30 - Sept. 3	Sept. 2 - 4
Species/Varieties	Panic grass / Rhodes grass / signal mix	Panic grass / Rhodes grass (uncoated) / signal mix	Panic grass / Rhodes grass (coated) / signal mix	Heritage Seeds Northern Evergreen Mix
Seeding rate (kg/ha)	3	3 - 5	3	3
Soil type	Deep sand (Karrakatta sand, grey sand over yellow sand)			
Insecticide				Applied with knockdown Danadim on Sept. 23 for RLEM and aphids Regeant on Nov. 9 for wingless grasshoppers

17.5. Success with kikuyu on the south coast

Producers: Morgan and Debbie Sounness
Property: 'Tamgaree'
Location: Gnowellen
Total area: 1277 ha
Arable area: 1123 ha
Crop area: 2008: 400 ha 2009: 290 ha 2010: 190 ha with 150 ha oats: and 40 ha field peas (pasture cropped)
Livestock enterprise: Self-replacing superfine merino flock
Livestock nos. Sheep 4950 total (1800 ewes, 1150 mature wethers, 1000 hoggets (ewe and wether), 1000 weaners)
Perennial pasture: 400 ha kikuyu
Annual rainfall: 425 mm

Morgan Sounness is an experienced kikuyu grower and seed producer. He sowed his first paddock to kikuyu in 1995 and now has 400 ha of kikuyu, with plans to sow more. Morgan also had 20 ha of perennial veldt grass sown in the early 80s, but this stand thinned out and was re-sown to kikuyu in 2008.

Morgan mentions multiple benefits to his farming enterprise from growing kikuyu:

- increased stocking rate
- better wool quality
- erosion control

- extended growing season and out-of-season green feed
- compatibility with annual legumes
- soil carbon improvement
- opportunity to crop perennial grass paddocks (pasture cropping).

Morgan uses a Great Plains 24-run no-till disc drill with double disc openers and trailing press wheels on a 7.5" (18.7 cm) row spacing. He changed the cogs to enable lower and more accurate seeding rates, which also eliminates the need for a fertiliser carrier to be used. Morgan added, "I would like to add some coulters and make the machine wider to speed up the job."

Morgan has made some changes to seeding over time, and has increased seeding rate from 1 kg/ha to at least 2 kg/ha. He adds, "Calibrate, calibrate, calibrate - the whole exercise is too expensive to get the seeding rate wrong."

Morgan states that weed control is absolutely critical for successful establishment. "The first paddock I sowed had much better success around the outside of the paddock, due to leftover herbicide being applied there. A key thing to avoid is leaving the clover and sowing kikuyu in to it. It never works – the clover out-competes the kikuyu every time. I now use Dicamba with the knockdown to achieve better control of subterranean clover and erodium."

Morgan says seeding depth is also critical, "I avoid top-dressing seed on the surface, as kikuyu must be sown with some covering of soil. The seeding depth now reflects soil moisture, and I will chase moisture at depth if needed, up to 25 mm."

I don't use any fertiliser at seeding, as this eliminates the risk of burning the seed and the initial root system."

"Spring sowing is the most reliable, but autumn sowing can work with kikuyu, especially if ryegrass isn't present. If ryegrass is present in the paddock, spray-topping the year before sowing kikuyu is advisable to reduce the seed bank."

Morgan gauges seeding success of kikuyu as an even germination across the whole paddock, which eliminates bare areas and erosion risk. "I assess the plant density across the paddock using a sliding scale, more than 5 plants/m^2 is excellent establishment, 1 to 5 plants/m^2 is good, while less than 1 plant/m^2 is acceptable," Morgan said.

Morgan's advice for wind erosion-prone sites is to sow French Millet at 1 kg/ha as a cover crop (but only for cattle producers – as sheep won't eat it and will then target the kikuyu), or to sow a winter cereal at <10 kg/ha. He also allows the annual pastures to bulk up more before the knockdown spray to provide better cover. Morgan says that he is very reluctant to spray out summer weeds that emerge with the kikuyu, as these provide protection from the wind.

Details of kikuyu sowing in 2008

Area	20 ha
Soil type	Sand over gravel
Paddock history	Long-term annual pasture (although originally a perennial veldt grass pasture)
Paddock preparation	Grazed hard through winter Sprayed with 1.5 L/ha Glyphosate + Cadence (label rate) in early August Sprayed with 1 L/ha SpraySeed + 200 ml/ha alpha-cypermethrin on Sept. 2
Weeds targeted	Mainly subterranean clover, capeweed, erodium
Date of seeding	First week of September
Species/Varieties	kikuyu (uncoated)
Seeding rate (kg/ha)	5 (as produces own seed, but would normally use 2 kg/ha)
Soil moisture at seeding	Dry at the surface, but moist just below
Insecticide	Just with the initial knockdown
How often do you check for insect pests?	Once every 2 weeks during the first 2 months post-seeding

18. Further reading

Perennial pastures for Western Australia, Bulletin no. 4690, Department of Agriculture and Food Western Australia

Identifying sub-tropical grass seedlings, Bulletin no. 4775, Department of Agriculture and Food Western Australia

Establishing sub-tropical perennial grasses, Farmnote No 443, Department of Agriculture and Food Western Australia

Sub-tropical grass options for the Northern Agricultural Region, Farmnote no. 445, Department of Agriculture and Food Western Australia

Sub-tropical grass options for the south coast, Farmnote no. 446, Department of Agriculture and Food Western Australia

Department of Agriculture and Food
GOVERNMENT OF WESTERN AUSTRALIA
Note: 443
September 2010
Farmnote

Establishing sub-tropical perennial grasses

By Phil Nichols, Ron Yates and Geoff Moore (Department of Agriculture and Food, Western Australia) and Phil Barrett-Lennard (agVivo/Evergreen Farming)

This Farmnote describes the key steps for establishing Rhodes grass, panic grass, signal grass, setaria, digit grass, bambatsi panic and kikuyu, either alone or in mixtures. The aim should be to establish a productive long-term pasture with a perennial grass density of at least 10 plants/m^2.

Plan ahead

Plan a year ahead and reduce weed seed-set by grazing and spray-topping, especially for difficult-to-control weeds. For exposed paddocks prone to wind erosion, consider sowing a cereal to provide stubble for soil stability. Commence control of rabbits and kangaroos if they are a potential problem.

Purchase good quality seed

Select species and varieties suited to your district, soil type and intended use. Refer to Farmnote 445 for best sowing options for the northern agricultural region and to Farmnote 446 for the south coast.

Germination of commercial sub-tropical grass seed batches typically varies from 10–60%. Kikuyu (commonly >80%) is an exception. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. Several seed companies coat their seeds. While this helps with handling and flow of fluffy seeds in machinery, it reduces the number of viable seeds per kilogram.

Seed dormancy—Panic grass, setaria and signal grass have a high proportion of seeds, referred to as *fresh seeds*, which remain dormant for 6–10 months after harvest. Consider purchasing these species the year before seeding and storing under dry conditions. Rhodes grass and kikuyu do not have such dormancy.

Control weeds and insects prior to sowing

A weed-free seed bed is essential, as sub-tropical grass seedlings are weak competitors. Weed control strategies should also aim to minimise wind erosion, particularly on exposed sites with sandy soils. One strategy is to use a selective broadleaf herbicide 6 weeks from sowing, followed by a general knockdown herbicide 2 weeks from sowing. This allows grass residues to bind the soil and reduce erosion risk.

In paddocks with a low grass density, a single knockdown herbicide 2 weeks before sowing can be used, but higher rates than autumn-winter applications will be needed to kill difficult-to-control weeds.

Two knockdown sprays (6 weeks and 2 weeks before seeding) result in good weed control but leave the paddock prone to wind erosion (unless stubbles have been retained). It also reduces the amount of winter grazing and the ability to change plans if sowing conditions deteriorate.

Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

Sow into moisture in late winter – early spring

The best sowing times for the main regions suited to sub-tropical grasses are:

- Dongara—Kalbarri districts—early to late August
- Perth—Eneabba districts—mid-August to early September
- South coast—early September to early October

This coincides with sufficient soil temperatures for germination and the likelihood of sufficient moisture for good root development before summer.

Set-up the seeder for the best result

Machinery and implements (tynes or discs) can vary but should have the following features.

Formation of furrows—Furrows capture rainfall and increase seedling survival, particularly in dry springs. Furrow formation also scalps away non-wetting sand and removes weed seeds. Furrows should be formed to minimize sand in-fill and sides should not be too steep. Furrows of 50 mm depth are sufficient if the soil surface is moist. Deeper furrows can be used if the soil surface is dry or highly non-wetting.

Press wheels—Press wheels provide good seed contact with soil moisture. They should press soil in the furrow bottoms and minimise sand in-fill from the sides. Rounded or flat-bottomed wheels give the best results.

Optimum row spacing—Optimum row spacing is 50–60 cm. This reduces soil movement into adjacent furrows and allows annual pastures to grow between rows in the cooler months. Wider row spacings can be used in low rainfall areas to reduce moisture competition.

Sowing Rhodes grass and panic grass in alternate rows prevents the more vigorous Rhodes grass dominating mixtures of the two species.

Sow 2–5 kg/ha of seed

Use a sowing rate of 2–5 kg/ha, depending on seed quality and whether seed is coated. For uncoated seed with >40% germination, 2 kg/ha is sufficient. Higher rates should be used for seed of lower germination. Higher rates are also required for coated seeds to sow the same number of seeds per area.

Sow at a depth of 5–10 mm

Sub-tropical grasses require shallow seeding to a depth of 5–10 mm. **Seeds sown too deep will not emerge**. A common method is to drop seeds in the bottom of furrows and press them below the surface with press wheels. Sowing directly onto the surface with no soil cover is unreliable.

Don't sow too fast

Sowing too fast causes excessive soil movement, reducing the accuracy of seed placement. Sand in-fill increases and furrows can collapse, causing deeper seed burial than intended. Speeds of 5–10 km/h generally give a good result. Check seed placement regularly and make adjustments if needed.

Control weeds and pests (insects, kangaroos and rabbits)

Summer growing weeds compete strongly with sub-tropical grass seedlings for soil moisture and their control will maximise establishment. However, if there is a major erosion risk they should be retained. Monitor the paddock for insect damage, especially over the first 6–8 weeks and control if needed. Good control of kangaroos and rabbits is essential to protect young sub-tropical grasses.

Defer grazing until grasses are well established

The first grazing of sub-tropical grasses should be deferred until they are well established and actively growing. This will vary with seasonal conditions and may not be until after the break of season. Ensure plants are firmly anchored before introducing animals.

Further information

Identifying sub-tropical grass seedlings, Bulletin No 4775

Sub-tropical grass options for the northern agricultural region, Farmnote 445

Sub-tropical grass options for the south coast, Farmnote 446
Perrenial pastures for Western Australia, Bulletin No 4690'

Acknowledgements

Funding for this work was provided by the Future Farm Industries CRC, Meat & Livestock Australia, Australian Wool Innovation Limited and the former Land and Water Australia.

20102646-09/10-ID10484

ISSN 0726-934X

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Direct seeding of old man saltbush

By Phil Nichols, Ron Yates and Ed Barrett-Lennard (Department of Agriculture and Food, Western Australia)

Establishing old man saltbush (*Atriplex nummularia*) to revegetate salt affected land and rangelands has become increasing popular. The most reliable way to establish old man saltbush is by transplanting seedlings, but this can cost more than $450/ha. A much cheaper method ($100-150/ha) has been to direct seed old man saltbush using a specialised niche seeder, but this has proved far less reliable. However, new research has developed a simpler and more reliable method for direct seeding of old man saltbush. This Farmnote describes nine key steps to establish old man saltbush by direct seeding, using modifications to conventional seeding machinery.

Experimental plots showing successful direct seeding of old man saltbush at Meckering

Select appropriate paddocks

Good site selection is critical to successful establishment. The most appropriate paddocks are those with slightly- to moderately-saline land (EC_e values at 0-30 cm depth between 2 and 8 dS/m). Productivity is markedly reduced on extremely saline land (EC_e > 32 dS/m). Duplex soils with sandy loam over clay are the easiest to establish, as the sandy surface allows salts to leach through the soil following winter rains, creating a favourable environment for germination.

Old man saltbush is susceptible to waterlogging, so avoid areas prone to prolonged waterlogging. Sites with 50-100% annual ryegrass and some slender ice plant are generally ideal. If the site has samphire or is bare and scalded, then it is too saline. If the site has substantial sea barleygrass, then it is too waterlogged.

Prepare sites for optimum establishment

A weed-free seed bed is essential, as old man saltbush seedlings are weak competitors. Plan a year ahead if possible and reduce weed seed-set in the year before sowing by grazing and spray-topping, especially for ice plant and annual ryegrass. Commence control of rabbits and kangaroos if they are a potential problem.

A good weed control strategy is to use two knockdown sprays (4-6 weeks and 1-2 weeks before seeding). Cultivation prior to sowing can make the surface too uneven for precise sowing.

Apply a residual insecticide with the final knockdown herbicide (or at sowing) to control caterpillars, cutworms, aphids and redlegged earth mites.

Sow the best seed

Germination of old man saltbush seed batches is generally low, and typically varies from 5–20%. Send to an accredited seed laboratory if concerned about the expected germination of a seed batch. The following steps will help maximise use of high seed quality.

Ensure seed is subspecies nummularia

There are two subspecies of old man saltbush. Subspecies *nummularia* is palatable to sheep and is best suited to direct seeding using the methods described in this Farmnote. The alternative subspecies, subsp. *spathulata*, is unpalatable to grazing sheep and will not readily establish from seed.

Ensure seed is fresh

Use seed harvested within the previous six months that has been stored in a cool, dry environment.

Use large heavy fruits

Large and heavy fruits should be selected for sowing, as they are more likely to contain mature, viable seeds. Small fruits, on the other hand, are more likely to be empty or contain undeveloped embryos. If there is a large range in fruit size, grade off the smaller fruits.

Retain bracts

Do not remove the bracts surrounding the fruit, as establishment success in the field has been higher for seeds with bracts, compared to seeds with their bracts removed.

Sow into moisture in late winter – early spring

Sow into a moist seedbed, or if there is a strong likelihood of rain. Although old man saltbush is salt tolerant, its germinating seeds are susceptible to salinity. Seeds in a saline environment will not germinate if the soil surface is too dry, as it is quite likely to be too saline. If the area is waterlogged, sowing should be deferred until later in spring, or to the following year.

The best sowing times for different regions in Western Australia are:

- Northern agricultural region - early to late August
- Central and eastern wheatbelt - mid-August to early September
- Southern agricultural region - late August to late September

This coincides with sufficient soil temperatures for germination and the likelihood of sufficient moisture for good root development before summer.

Aim to establish at least one plant per 2 m of row

Use a sowing rate of ~10 fruits/m (if germination rate is 15%). This should provide at least one plant every 2 m. Higher rates should be used for seed of lower germination.

Set-up the seeder for the best result

A standard Massey Ferguson combine seeder (or something similar) can be used to direct seed old man saltbush, with some minor modifications. The simplest way is to remove tynes not needed for sowing. Small boxes (one for each seeding tyne) can be attached to the seeding box to hold the small quantity of seed required, with hoses attached to seeding tynes. Seeders should also have the following features.

Formation of furrows

Tynes should create furrows to capture rainfall and increase seedling survival, particularly in dry springs. Furrow formation also scalps away non-wetting sand and removes weed seeds. Furrows should be formed to minimise soil in-fill and should be broad to increase the area of rainfall capture.

The key is to sow into moist soil. Furrows of 50 mm depth are sufficient if the soil surface is moist, while deeper furrows can be used if the soil surface is dry or highly non-wetting, provided furrow collapse and soil in-fill is avoided, as this causes burial of the seed, which can markedly reduce seedling emergence.

Press wheels

Press wheels provide good seed contact with soil moisture and reduce in-fill of soil into the furrows. They should press soil in the furrow bottoms and minimise soil in-fill from the sides. Flat-bottomed wheels give the best results.

Row width

Calculate the desired spacing between saltbush rows for each seeding pass and adjust the width of seeding tynes accordingly, removing non-seeding tynes. Typical row widths for alleys of double or triple rows are 1 m.

Sow at a depth of 5–10 mm

Old man saltbush requires shallow seeding to a depth of 5–10 mm. **Seeds sown too deep will not emerge**. The simplest method is to drop fruits in the bottom of furrows and press them in with press wheels. When correctly adjusted, this will leave a small proportion of fruits visible on the soil surface. Sowing directly onto the surface is unreliable.

Control weeds and pests (insects, kangaroos and rabbits)

Summer growing weeds compete strongly with saltbush seedlings for soil moisture. Control weeds with appropriate herbicides to maximise establishment. Monitor the paddock for insect damage, especially over the first eight weeks and control if needed. Good control of kangaroos and rabbits is essential to protect young seedlings.

Defer grazing until seedlings are well established

The first grazing of old man saltbushes should be deferred until they are well established and actively growing. This will vary with seasonal conditions and may not be until after the break of the next season. Ensure plants are firmly anchored before introducing animals.

Further information

Barrett-Lennard, E.G. (2003). Saltland pastures in Australia – a practical guide 2nd ed. (Sustainable Grazing on Saline Lands Program, Canberra).

Acknowledgements

Funding for this work was provided by the Future Farm Industries CRC, Meat & Livestock Australia, Australian Wool Innovation Ltd and the former Land and Water Australia.

Recruitment of desirable perennial grass seedlings within existing pastures

Roshan Thapa and David Kemp, Charles Sturt University, Orange, New South Wales

This bulletin aims to provide farmers and their advisors with a simple low-cost pasture management strategy to encourage emergence of new seedlings (*i.e.* seedling recruitment) of desirable perennial grasses within existing pastures in the 600 - 800 mm rainfall zone of south-eastern Australia. Although it is mainly aimed at native pastures containing desirable perennial grasses, the principles can also be applied to sown perennial grass pastures.

Principles

Allowing new seedlings of desirable perennial grasses to establish within existing swards is a viable practice that farmers can use to restore pastures to a more productive state (~60% desirable perennial grasses), without the need for re-sowing. The investment costs are considerably less and restored paddocks are more profitable over time.

Work with one or two paddocks at a time, rather than the whole farm, in the first instance. Start by selecting a paddock with a moderate content of desirable perennial grasses (around 20-30% is ideal), rather than the poorest paddock. Better paddocks provide faster rates of improvement and quick seed production. The seeds are then available for harvesting and redistribution to poor paddocks.

The paddock has to be free of major weed problems. Control annual grasses and seasonal weeds, with herbicides and grazing, in the year before pasture improvement is attempted. Desirable perennial grasses can tolerate low rates of herbicide applications. Having a paddock relatively free of weeds is a prerequisite for success of this management strategy.

Few seeds of perennial grasses generally remain in the soil from previous seasons. Therefore, emergence of new seedlings depends predominately on seeds set in the current year. Grazing needs to be managed to encourage flowering and seed set.

Rest paddocks (i.e. remove stock) to maximise flowering and seed set creates sward conditions that enhances emergence of seedlings. Standing plant material (even if ~80% dead) is more useful than litter (dead plant material lying on the soil surface) in most instances. More than 2000 DM kg/ha of litter is detrimental to emerging seedlings.

Determine basic soil characteristics of the paddock, including nutrient status, pH and texture. Fertilisers should be applied to address nutrient deficiencies that affect seedling growth, especially in low fertility areas. Phosphorus, in particular, is important for early root growth of seedlings. Roughing up the soil surface by harrowing, especially on soils that are hard setting, increases the locations where seeds can lodge and germinate, thus enhancing emergence of new seedlings.

Consider use of an insecticide to control seed harvesting ants if they are a problem. This is often the case with phalaris in sown pastures.

Rainfall events in late summer or early autumn that result in at least 10 days of moist soil in the top 50 mm triggers successful seedling emergence -15 days of surface soil moisture is ideal. Seedling emergence is not hindered by 2 days where the soil surface is dry during this period.

Some establishment of new seedlings will occur in most years, but resting paddocks from late spring through to late March and following the management strategies in this bulletin will maximise the opportunities for seedling emergence.

In the first year of applying these management tactics, a conservative rate of improvement in perennial grass content may be 0-20% of the total herbage mass produced per year. It could take

4-5 years to reach a target of 60% desirable perennial grasses, if there is an average of 10% improvement per year.

Figure 1 shows the steps involved for enhancing seedling establishment of desirable perennial grasses within existing swards

Seasonal management tactics

Spring

Do not let the selected paddock get overgrown in early spring. Aim to have the herbage mass around 1500-2000 kg/ha of dry matter when the paddock is locked up in late spring to allow plants to flower and set seed.

Early – mid summer

Allow seeds to mature and fall onto the soil surface. Monitor seasonal conditions in December and January. Continue the summer rest if rainfall for these months is >40% of average rainfall.

If the season is poor (<40% average rainfall in these months), defer setting up the paddock for seedling establishment until the next year. Even if rains in late February provide the right conditions for recruitment, seed set will have been minimal and feed will be needed for livestock.

Consider applying an insecticide if seed harvesting ants are present in high numbers. Phalaris is of the most concern.

On hard-setting soils carry out mild soil disturbance to provide micro-environments for seeds to lodge and germinate. This can be done using harrows dragged behind a vehicle.

Late summer - early autumn

Look for new perennial grass seedlings within 2-3 weeks of a significant rainfall event in late February or March.

Autumn - winter

Once seedlings cannot be pulled out easily by hand, the paddock can be lightly grazed until ~1500 kg/ha of dry matter remains.

Guidelines developed for managing sown pastures are also applicable to managing pastures enhanced through natural establishment.

Benefits

Costs involved in improving paddocks through natural seedling recruitment will be substantially less than re-sowing a new pasture, as few inputs are required. In a good season resting one or two paddocks will have little impact on the feed supply needed to carry livestock on the farm. Soil disturbance using harrows may cost ~$20/ha and is only necessary for hard setting soils. Insecticides and herbicides will incur some additional costs if they are needed. However, this will be considerably less than the ~$300/ha needed to sow a new pasture.

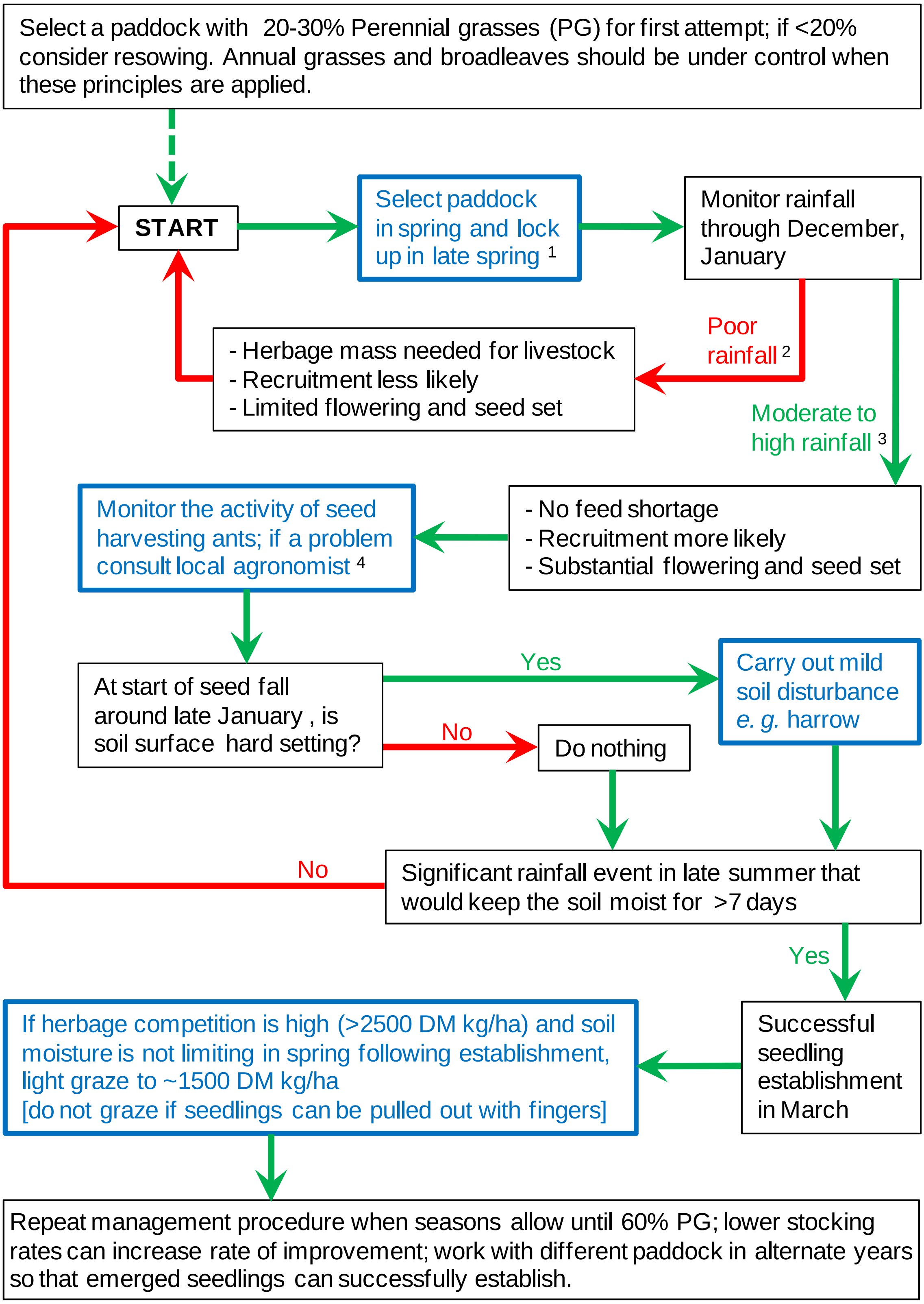
Once a stable target of 60% content of desirable perennial grasses is reached, increased livestock production will result, either through greater carrying capacity or more production per head. Estimates for some native grass pastures in New South Wales suggest livestock production can be more than doubled in the medium term, depending on soil characteristics.

It is useful for farmers to rest some paddocks to enable seedling establishment if suitable seasonal conditions prevail. If the conditions turn dry over summer then emergence of new seedlings is less likely. Rested paddocks can then be grazed, as feed is likely to be limited at this time. In wet summers there is usually no shortage of feed and resting paddocks for longer periods is not likely to cause major difficulties.

This information now provides farmers with a low-cost approach to pasture improvement for those large parts of the landscape where there are still some desirable perennial grasses.

Acknowledgements

Roshan Thapa conducted much of this work through a PhD project funded by Meat & Livestock Australia. This work also formed part of a project through the Future Farm Industries CRC, with additional funding provided by Meat & Livestock Australia, Australian Wool Innovation Ltd and Land & Water Australia. Additional information was provided by Colin Langford, David Michalk, Fiona Leech, Linda Ayres, Lori McWhirter and Warwick Badgery (all from Industry & Investment NSW,) and Phillip Nichols (Department of Agriculture and Food Western Australia)



Notes:

[1] If dry spring, forego the option of fostering recruitment as flowering and seed set will be limited over summer

[2] Poor rainfall equates to less than 40% of average rainfall for December-January

[3] Moderate rainfall equates to at least 40% of average rainfall for December-January

[4] Ants are of the most concern with phalaris while they are less of a problem with native species

Colour codes: Green = Moving forward; Red = Exit points; Blue = Actions to be done in the field

Figure 1: Decision support pathways to enable seedling establishment of desirable perennial grasses within existing swards.

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Enhancing the germination of three fodder shrubs (*Atriplex amnicola*, *A. nummularia*, *A. undulata*; Chenopodiaceae): implications for the optimisation of field establishment

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Abstract. Saltbush (*Atriplex*) species are widely grown in Australia as saltland pastures. Direct seeding practices for saltbush currently result in asynchronous and unreliable seedling establishment (5% successful establishment is not uncommon from field-sown seed). In part this may stem from a limited understanding of *Atriplex* seed germination requirements. This paper presents findings with 3 *Atriplex* species, *A. amnicola* (Paul G. Wilson.), *A. nummularia* (Lindl.), and *A. undulata* (D. Dietr), each of which differs in germination characteristics. For *A. amnicola*, the presence of light (and artificial substitution of light by 1000 ppm gibberellic acid) improved germination under controlled conditions and resulted in a 4-fold increase (70% total emergence) in field emergence of seedlings. For *A. undulata*, removing bracteoles increased germination under controlled conditions (∼15%), with a 1.5-fold improvement in field seedling emergence (55% final emergence); however, seed priming or gibberellic acid application had no significant effect. In contrast, for *A. nummularia*, bracteole removal and light had minor positive effects on germination under controlled conditions, but this did not translate into improved emergence in soil or in the field. Under −0.5 MPa NaCl stress, application of gibberellic acid, salicylic acid, or kinetin to the germination medium significantly increased the final germination percentage of *A. amnicola* seeds (58, 16, and 14%, respectively) and improved the rate at which seeds germinated. All plant signalling compounds significantly increased final germination percentage and germination rate of *A. undulata*, albeit with a <10% increase at −0.5 MPa NaCl. Priming seeds with plant signalling compounds had similar effects on seed germination under low water potentials compared to direct treatment of the germination media. The effects of seed priming on *Atriplex* seedling emergence from saline soils varied among species. Priming with water significantly increased emergence percentage of *A. amnicola* but had no effect on *A. nummularia* and *A. undulata*. Gibberellic acid improved *A. amnicola* germination parameters only, whereas salicylic acid and kinetin improved the rate of emergence in all 3 species at various levels of salinity. This study suggests that a basic understanding of seed dormancy and germination requirements has the potential to substantially improve field emergence of saltbush species.

Additional keywords: gibberellic acid, kinetin, salicylic acid, salinity, saltbush, seed dormancy.

Introduction

Much of the Australian agricultural landscape is becoming salt affected. It is estimated that ∼5.7 Mha of agricultural land are currently at risk of salinity, with estimates of ∼17 Mha over the next 50 years being at risk of soil salinity (NLWRA 2001). Saltland pastures composed at least partly of saltbush species (*Atriplex* spp.) have been a recommended response to salinity for more than 60 years (Teakle and Burvill 1945). These pastures improve farm profitability in Mediterranean (winter rainfall dominant) farming systems by providing supplemental feed sources in summer and autumn when other sources of paddock feed are less available (Barrett-Lennard *et al*. 2003).

The Australian native *Atriplex amnicola* and *A. nummularia*, and the Argentinean species *A. undulata* are widely used in saltland pasture development in Australia (Barrett-Lennard *et al*. 2003). These species can be established from mixtures of seed sown with a specialised 'niche seeder' (Malcolm and Allen 1981) or as nursery-raised seedlings planted with a commercial tree-planter (Barrett-Lennard *et al*. 1991). The dilemma facing farmers wishing to establish saltland pastures is the

 10.1071/AR06031 0004-9409/06/121279

trade-off between cost and risk: niche seeding is relatively cheap to undertake (~\$150/ha) but has a higher inherent risk of failure; in contrast the planting of nursery-raised seedlings has a lower risk of failure, but is far more costly (~\$450/ha) (Barrett-Lennard *et al.* 1991).

With *A. amnicola*, there are indications that exposing seed on the soil surface can result in substantial establishment failure, particularly if rainfall events in the first spring after sowing are not sufficiently frequent to maintain seedling water relations during radicle emergence and penetration into the soil (Vlahos 1997). On the other hand, burial of seed (which presumably results in more favourable seed water-relations) can also be associated with substantially decreased rates of establishment (Vlahos 1997). This is clearly a dilemma. The research in this paper is part of a wider investigation to improve methods of establishing saltbush-based saltland pastures using direct seeding. In the present work we focus on means of physical and chemical manipulation of the seed to improve germination performance and reliability.

Atriplex seeds are enclosed within bracteoles that range in form from thin membranous to heavily lignified, robust structures (Garvin and Meyer 2003), which ecologically may serve to control timing of seed germination and aid in dispersal (Ungar and Khan 2001). Restriction of *Atriplex* seed germination by bracteoles is thought to occur through a number of mechanisms including mechanical inhibition (Young *et al.* 1980), ionic or osmotic stress (Beadle 1952; Mandak and Pysek 2001), negative effects of growth regulators/production of allelopathic substances (Askham and Cornelius 1971; Jefferson and Pennacchio 2003), reduction of oxygen movement to the seed (Barrett-Lennard and Gavelle 1994), and reduction of cold stratification of the seed (Garvin and Meyer 2003). Removing bracteoles from *Atriplex* seed does not necessarily remove primary dormancy (Garvin and Meyer 2003) but may increase germination vigour (Springfield 1964) and result in improved plant establishment (Malcolm 1972).

Atriplex species are halophytes and are distinguished from glycophytes by their higher tolerance to saline conditions, regulated by enhanced ion control mechanisms (Munns *et al.* 2002). The presence of these salt-tolerance mechanisms varies throughout plant development, with salinity tolerance in mature plants being independent of that at germination (Foolad 1999); typically, germinating seeds have lower stress tolerance than seedlings and mature plants (Dodd and Donovan 1999). Increasing soil salinity leads to a reduction or delay in germination of both glycophytic and halophytic plants. However, rainfall events may increase soil water potential and allow for subsequent successful seed germination.

Given the harsh micro-environment in which *Atriplex* species grow, often being characterised by high soil salinity and drought, there is a requirement for seed treatments to be developed to maximise the potential of the seed to germinate and emerge rapidly (Powell 1998). Previous studies have investigated treatment of *Atriplex* seed involving removal of bracteoles (Ungar and Khan 2001; Garvin and Meyer 2003); this paper expands on these studies. Seed priming techniques may result in more rapid seed imbibition, increased extensibility of radicle cell walls, and endosperm weakening, which may reduce the lag phase prior to radicle emergence, increasing seedling vigour (Twitchell 1955; Haigh 1988). Here we study the effects of priming seeds with the plant signalling compounds gibberellic acid (GA_3), kinetin, and salicylic acid to (*a*) overcome non-deep physiological dormancy, a characteristic of *Atriplex* species (Baskin and Baskin 1998), and (*b*) improve abiotic stress tolerance in the germinating seed (Zhang *et al.* 1994; Khan *et al.* 2003, 2004) or resultant seedling (Aldesuquy *et al.* 1998; Senaratna *et al.* 2000; Ashraf and Rauf 2001; Harris *et al.* 2001) thereby increasing seedling vigour under stressful growing conditions.

Materials and methods

Seed characteristics

Intact fruits of 3 saltbush species (*Atriplex* spp.) (*A. amnicola* Paul G. Wilson., *A. nummularia* Lindl., and *A. undulata* D. Dietr.), were obtained from a local seed merchant, Kimseed Pty Ltd, Western Australia, in December 2004. Laboratory and glasshouse experiments were conducted between February and July 2005 on seed stored dry at ambient laboratory conditions (*c.* 23°C).

The chartaceous bracteoles were removed from the seeds by gently rubbing the intact, dried fruits between sheets of corrugated rubber matting. Seeds were removed from the resultant material by sieving. Seed damage as a result of the cleaning process was minimal when the seed coats were carefully inspected under a dissecting microscope, and germination experiments confirmed no difference in germination rate or final germination percentage between hand- and rubber-mat-extracted seed.

General experimental conditions

The experiments in this study were conducted in 2 parallel streams. The first stream focussed on the effects of bracteole removal, exposure to light, and priming with GA_3 as a means of increasing germination and overcoming the inhibition in establishment with seed burial. The second stream focussed on germination under NaCl- or PEG_{6000}-induced water deficits, and the capacity of seed priming with plant signalling compounds to overcome inhibitory effects.

For most germination experiments, 4 replicates of 25 seeds were germinated in 90-mm Petri dishes containing one 84-mm germination paper (Type: 424, Advantec, Toyo Roshi Kaisha Ltd, Japan) moistened with 7 mL of test solution. Petri dishes were sealed with Parafilm and incubated at 18/5°C (TLMRIL model, Thermoline, Qld, Australia), unless stated otherwise. The higher temperature coincided with the 12-h light period (400–700 nm, 16.3 μmol/m^2.s) and lower temperature coincided with the 12-h dark period. This temperature range is representative of winter–early spring temperatures in Western Australia when *Atriplex* species normally germinate. Seeds were considered to be germinated with the emergence of the radicle, provided subsequent root growth was observed. Germinated seeds were scored under low-light conditions to restrict light interactions with germination, and removed from the Petri dish.

Experimental stream 1: bracteole removal, light effects, and seed priming with GA_3

Germination and bracteole removal

To determine the role of bracteoles in germination, *Atriplex* seeds were either sown: (*a*) as intact fruits with bracteoles attached, (*b*) with bracteoles removed, or (*c*) with bracteoles removed and seeds placed on top of a 1-mm layer of ground (<500 μm) bracteole material. Seeds were germinated in the dark (Petri dishes wrapped in aluminium foil) and % germination was measured every alternate day for 14 days. *Atriplex* seed germination performance was assessed by calculating final germination percentage and a germination rate index (GRI, Eqn 1):

$$\mathrm{GRI}\ (\%/\mathrm{day}) = \sum[(\mathrm{G}_i - \mathrm{G}_{i-1})/i] \quad (1)$$

where i is the germination count day, G_i is the percentage of seeds germinated at time i, and G_{i-1} is the percentage of seeds germinated the previous count day (Maguire 1962; Emmerich and Hardegree 1990). The germination rate index provides a measure of germination vigour.

Germination, light, and GA_3

Four Petri dishes moistened with deionised water, containing 25 seeds with bracteoles removed of *A. amnicola*, *A. nummularia*, and *A. undulata*, were placed in specially manufactured light-excluding chambers (20 by 20 by 15 cm) with windows made from Kodak Wratten filters exposing seeds to defined wavelength radiation. Seeds were germinated in light of the following characteristics: white (400–700 nm, 19.7 μmol/m^2.s), far-red (720 nm, 0.09 μmol/m^2.s), red (640 nm, 4.92 μmol/m^2.s), yellow (570 nm, 19.64 μmol/m^2.s), blue (475 nm, 2.38 μmol/m^2.s), or darkness. The chambers were placed in a constant 18°C (12/12 h day/night) incubator. Germination was monitored for 14 days.

The species that was determined to have a light requirement for germination from the above experiment (*A. amnicola*) was treated with an exogenous application of GA_3 (Progibb 100 g/L GA_3, Valent Biosciences, a division of Sumitomo Chemical Aust. Pty Ltd, Chatswood, NSW, Australia). In some light-requiring Western Australian species, exogenous GA_3 application has replaced the germination requirement for red-wavelength light (Plummer and Bell 1995). Four Petri dishes were moistened with either 7 mL of deionised water or 1000 ppm GA_3. Each dish contained 25 seeds with bracteoles removed and was placed in continuous darkness or in 12 h light (16.3 μmol/m^2.s) 12 h dark. Germination was monitored for 14 days.

Seed priming and germination enhancement

Seeds of *A. amnicola* with bracteoles removed were either not primed (control) or primed in deionised water or 1000 ppm GA_3. The optimal priming time for *Atriplex* seeds was 18 h, with longer priming times resulting in germination during the treatment and shorter periods ineffectively transferring the GA_3 chemical signal. Seeds were primed in solutions for 18 h either in continuous dark or in light (50.7 μmol/m^2.s) at 18°C. Seeds were then removed, blotted, and dried back to initial water contents (determined by weight) at 18°C in the dark for approximately 4 days. Seeds were then germinated at 18/5°C in the dark and germination was monitored for 21 days.

Seed burial

Soil was collected from the top 0.10 m of a mildly saline sandy loam profile from Meckering, Western Australia, in April 2005 to determine if results in controlled laboratory environments could be transferred to soil. Soil electrical conductivity and acidity were determined in a 1 : 5 soil : water mixture ($EC_{1:5}$ 2.56 mS/cm, TPS 900-C Conductivity Meter Australia; pH 7.03, TPS pH cube Australia). Soil was air-dried and sieved (<2 mm) prior to potting into free-draining pots (60-mm diam. by 80 mm). Seeds of *A. amnicola*, *A. nummularia*, and *A. undulata* were either not primed or primed in 1000 ppm GA_3 in the dark at 18°C for 18 h (as above). Four replicates of 25 seeds were sown by hand on the soil surface and precisely covered with either 0.005 m or 0.010 m of soil and placed in 23/8°C, 12/12 h day/night. Pots were lightly watered daily. Seedling emergence was monitored every alternate day for 28 days and calculations were made of final emergence and emergence rate index, determined as for the germination rate index calculations.

Field trial

A field trial was established at Meckering, WA, in September 2005 to observe effects of bract removal, seed priming, and GA_3 application on emergence of *A. amnicola*, *A. nummularia*, and *A. undulata* seedlings. The field soil was the same soil used in previous experiments (see *Seed burial*), $EC_{1:5} = 2.56$ mS/cm. Each species was either sown as intact fruits or with bracteoles removed. Seed with bracteoles removed was either not primed (control) or primed for 18 h in water or 1000 ppm GA_3. Seeds (50 per treatment) were sown by hand at an average depth of 0.008 m (±0.002 m) over a 0.25 m distance, with 5 replicates per treatment. Emergence was monitored twice weekly for 28 days. Temperature and rainfall were recorded over this period, with average day and night temperatures being 19.2 and 4.4°C, respectively, and total rainfall being 42.2 mm.

Experimental stream 2: water deficits and seed priming with plant signalling compounds

Germination of Atriplex *seeds at different water potentials*

The effect of water potential, an influential factor of seed germination particularly in saline conditions, on *A. amnicola*, *A. nummularia*, and *A. undulata* germination was investigated by supplementing germination media with either polyethylene glycol (PEG_{6000}) or NaCl. Osmolytes were added to germination paper equating to iso-osmotic potentials of 0, −0.25, −0.5, −0.75, −1, −1.5, or −2 MPa. The osmotic potentials of PEG_{6000} or NaCl were calculated by Eqns 2 and 3, respectively, where x is the concentration of PEG_{6000} by %w/v (Michel and Kaufmann 1973) or the concentration of NaCl (mM). *Atriplex* seed germination performance was assessed by calculating final germination percentage and a germination rate index:

$$\Psi_{\mathrm{PEG}}\ (\mathrm{MPa}) = -7.6049x^2 - 33.025x + 4.83 \quad (2)$$

$$\Psi_{\mathrm{NaCl}}\ (\mathrm{MPa}) = -0.0045x - 0.0218 \quad (R^2 = 0.9981) \quad (3)$$

Germination under saline conditions with plant signalling compounds

To improve germination vigour of *A. amnicola*, *A. nummularia*, and *A. undulata* under various saline conditions (0, −0.5, −1, or −1.5 MPa NaCl), various plant signalling compounds including 1000 ppm GA_3, 0.05 mM kinetin (Khan *et al*. 2004), and 0.5 mM salicylic acid (Senaratna *et al*. 2003) were examined. Salicylic acid was dissolved in 1 mL of 100% ethanol prior to adding to 1 L of water (Williams *et al*. 2003). Four replicates of 25 seeds with bracteoles removed were placed on germination papers moistened with one of the plant signalling compound/NaCl combinations. Seeds were germinated in the dark and monitored for 14 days.

Seed priming under saline conditions with plant signalling compounds

Treatments that resulted in the most significant improvement in germination under NaCl-induced water stress identified above were subsequently assessed as potential seed-priming agents. Main and interaction effects of priming agents were determined for seed germination for *A. amnicola* (1000 ppm GA_3, 0.05 mM kinetin, 0.5 mM salicylic acid) and for *A. nummularia* and *A. undulata* (0.05 mM kinetin, 0.5 mM salicylic acid). Seeds were primed for 18 h at 18°C in darkness. Four replicates of 25 seeds with bracteoles removed were placed on germination papers moistened with PEG_{6000} equating to an iso-osmotic solution of 0, −0.5, or −1 MPa (as above). Seeds were germinated in the dark at 18/5°C and monitored for 14 days.

Bracteole removal, seed priming, and seedling emergence from saline soils

Soil was collected from the top 0.10 m of the profile from Meckering, WA (sandy loam, pH($H_2$0) 7.03), in October 2005. Soil was collected from 3 locations varying in salt concentration (site 1, $EC_{1:5} = 2.56$ mS/cm; site 2, $EC_{1:5} = 3.82$ mS/cm; site 3, $EC_{1:5} = 4.81$ mS/cm). Soil was air-dried and sieved (<2 mm) prior to potting into free-draining pots (60-mm diam. by 80 mm).

Fruits of *A. amnicola*, *A. nummularia*, or *A. undulata* were either sown intact or with their bracteoles removed. For seeds with bracteoles removed, seeds were either not primed or primed in 1000 ppm GA_3 (*A. amnicola* only), 0.05 mM kinetin, or 0.5 mM salicylic acid in the dark at 18°C for 18 h. Four replicates of 25 seeds were sown at 0.010 m depth and placed in 23/8°C (12/12 h day/night). Pots were lightly watered daily to maintain surface moisture levels. Seedling emergence was monitored every alternate day for 14 days and final emergence and emergence rate index were determined in a similar manner to the germination rate index calculation.

Statistical analysis

Germination and emergence data (final percentage and rate index) were analysed by one-way analysis of variance. Percentage values were arc-sine transformed prior to analysis, although untransformed data are presented. Fisher's comparison of means was used to determine differences between treatments. Significance in the results section refers to a difference at the 5% level ($P < 0.05$).

Results

Experimental stream 1: bracteole removal, light effects, and seed priming with GA_3

Germination and bracteoles

Removing bracteoles or placing threshed seed on top of a 1-mm layer of bracteole material significantly increased germination rate index and final germination percentage in all 3 *Atriplex* species compared with intact fruits (Fig. 1). A significant reduction in germination rate index was observed in *A. amnicola* when seeds were placed on bracteole material compared with germination in the absence of bracteole material (Fig. 1). Although removal of bracteoles enhanced germination, final germination percentages for *A. amnicola* and *A. nummularia* with bracteoles removed remained relatively low (~60 and ~40%, respectively; Fig. 1). In 2 of the species (*A. nummularia* and *A. amnicola*), bracteole removal resulted in the same germination as in the presence of bracteole material after removal (Fig. 1).

Germination, light, and GA_3

The 3 *Atriplex* species responded differently to the presence of light, with white light increasing *A. amnicola* and *A. nummularia* final germination percentage 63 and 12%, respectively, and having no significant effect on *A. undulata* germination (Fig. 2). The application of white light to

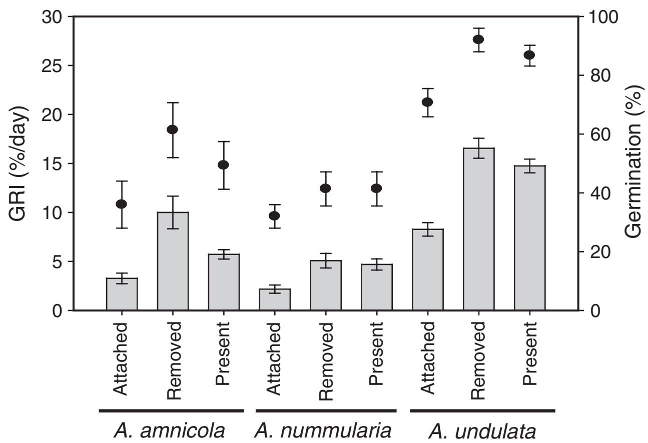

Fig. 1. The effect of bracteoles on germination rate index (bars) and final germination percentage (circles) on 3 *Atriplex* species with bracteoles attached, removed, or present (seeds placed on top of a 0.001-m layer of bracteole material). Values are means ± s.e. ($n = 4$).

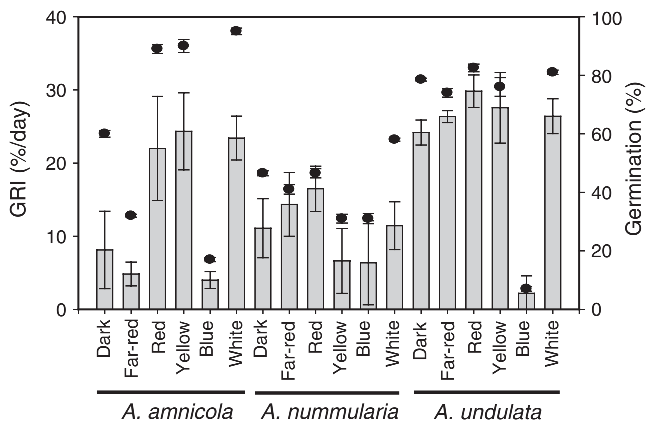

Fig. 2. The effect of light quality on germination rate index (bars) and final germination percentage (circles) on 3 *Atriplex* species grown in light-excluding chambers at 18°C. Chambers had either dark, far-red light (720 nm), red light (640 nm), yellow light (570 nm), blue light (475 nm), or white light (475–720 nm). Values are means ± s.e. ($n = 4$).

A. amnicola produced exceptional germination (99%, Fig. 2). Germination also significantly increased in *A. amnicola* when seeds were exposed to yellow (570 nm) or red (640 nm) light, which was not observed in *A. nummularia* or *A. undulata* (Fig. 2). Blue light (475 nm) significantly decreased final germination percentage in all *Atriplex* species compared with germination in the dark and was most pronounced in *A. undulata*, having a 71% reduction (Fig. 2). A strong positive linear relationship existed between final germination percentage and germination rate index ($R^2 = 0.89$).

Providing *A. amnicola* seeds with exogenous GA_3 replaced the light requirement for enhanced germination (Fig. 3). Seeds that received GA_3 in the dark had similar germination traits to seeds that received light. This equated

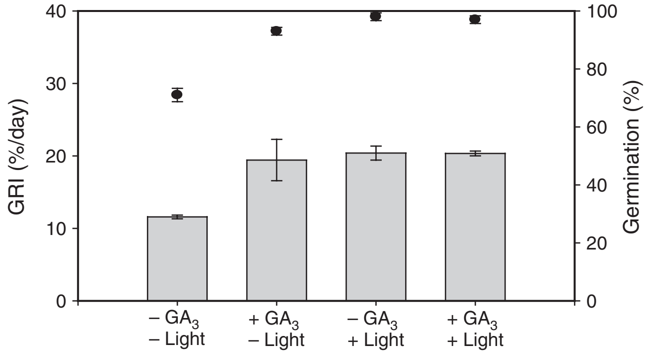

Fig. 3. The effect of exogenous application of 1000 ppm GA_3 and light (12 h, 16.3 µmol/m^2.s) on *A. amnicola* germination rate index (bars) and final germination percentage (circles). Values are means ± s.e. ($n = 4$).

to a 23% higher final germination percentage and a 67% higher germination rate index than untreated seeds in the dark (Fig. 3).

Seed priming and germination enhancement

Priming seeds in GA_3 significantly increased final germination percentage in *A. amnicola* by ~20% (Fig. 4), which was independent of light conditions (Fig. 3). Priming in water had no significant effect on final germination percentage of *A. amnicola* compared with unprimed seeds (Fig. 4). All priming treatments significantly improved the germination rate index of *A. amnicola* compared with the unprimed seed, ranging from a 27% increase in water-primed seed to a 72% increase with GA_3 (Fig. 4). Priming seeds in continuous light had no significant effect on germination rate index or on final germination percentage compared with priming in the dark (data not shown).

Seed burial

In *A. amnicola*, removing bracteoles from seeds had no effect on emergence parameters (Fig. 5). However, priming seeds with GA_3 prior to burial significantly increased the emergence rate index (5 times) and final emergence percentage (4 times) compared with non-primed seeds (Fig. 5). The depth in which seeds were buried had no significant effect on *A. amnicola* seedling emergence, with seeds buried to 0.010 m emerging to the same degree as seeds buried to 0.005 m (Fig. 5).

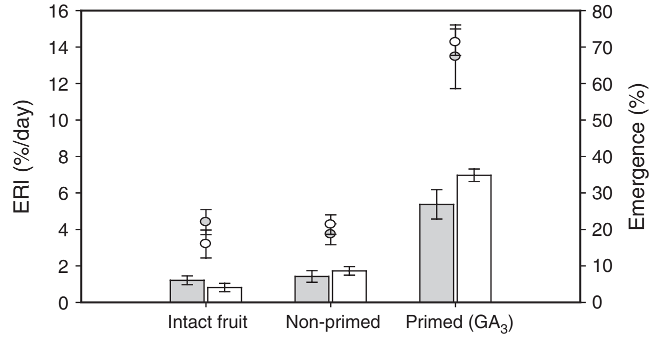

Fig. 5. Effect of bract removal and seed priming with 1000 ppm GA_3 on subsequent *A. amnicola* emergence rate index (bars) and final emergence percentage (circles) of seed buried in soil at either 0.005 m (grey) or 0.010 m (white) depth. Values are means ± s.e. ($n = 4$).

Field trial

Results from the field trial were consistent with results observed from previous experiments. Intact fruits of both *A. amnicola* and *A. undulata* had the lowest emergence percentage and germination rate index of all the treatments, with removing the bracteoles significantly improving emergence traits 50 and 150%, respectively (Fig. 6). Priming seeds of *A. amnicola* significantly improved emergence parameters, with priming in GA_3 giving 60% emergence

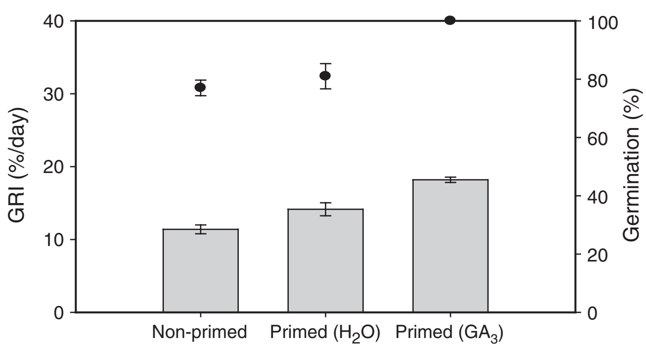

Fig. 4. Effect of seed priming at 18°C in the dark with water (H_2O) or 1000 ppm gibberellic acid (GA_3) on subsequent *A. amnicola* germination rate index (bars) and final germination percentage (circles). Seed germination occurred in the dark at 18/5°C. Values are means ± s.e. ($n = 4$).

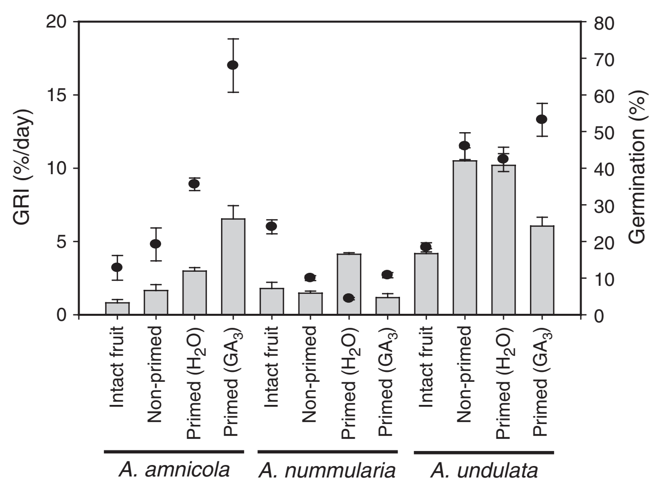

Fig. 6. Effect of bract removal and seed priming with water (H_2O) or 1000 ppm GA_3 (GA_3) on subsequent field emergence rate index (bars) and final emergence percentage (circles) of 3 *Atriplex* species buried at ~0.008 m depth. Values are means ± s.e. ($n = 5$).

and an average emergence rate of 7.5%/day compared with water with 25% emergence and an average emergence rate of 2.5%/day (Fig. 6). Priming had no significant effect on *A. undulata* emergence parameters, with bracteole removal providing the largest increase in emergence (Fig. 6). Field emergence of *A. nummularia* remained low, with intact fruits having the highest emergence (20%) (Fig. 6).

Experimental stream 2: water deficits and seed priming with plant signalling compounds

Germination of Atriplex *seeds at different water potentials*

Maximum final germination percentage of all *Atriplex* species occurred at 0 MPa (Fig. 7*a*). Germination of *A. nummularia* and *A. undulata* was less sensitive to lower water potentials than *A. amnicola*, with *A. amnicola* having a 50% reduction in germination percentage at ~–0.25 MPa compared with ~–0.70 MPa for *A. undulata* and ~–0.75 MPa for *A. nummularia* (Fig. 7*a*). Germination rate index was significantly decreased at –0.25 MPa in all *Atriplex* species compared with 0 MPa (Fig. 7*b*).

Germination under saline conditions with plant signalling compounds

All plant signalling compounds significantly increased final germination percentage and germination rate index of *A. amnicola* under non-saline conditions, with GA_3 having the most pronounced effect (27% increase, Fig. 8*a*). At −0.5 MPa NaCl, GA_3, salicylic acid, or kinetin application to *A. amnicola* seeds significantly increased the final germination percentage (by 58, 16, and 14%, respectively) and also increased the germination rate index. At –1 MPa NaCl, germination was significantly reduced in all treatments and, despite GA_3 significantly increasing final germination percentage compared with the control, only 12% of seeds germinated (Fig. 8*a*).

The application of plant signalling compounds to *A. nummularia* had no significant effect on final germination percentage under control (0 MPa) or −0.5 MPa conditions despite lower mean germination in salicylic acid-treated seed at 0 MPa. Kinetin and salicylic acid improved germination rate index at −0.5 MPa (Fig. 8*b*), with kinetin also improving germination rate under control conditions.

The application of kinetin and salicylic acid to seed of *A. undulata* had no significant effect on final germination percentage or germination rate index under non-saline conditions (Fig. 8*c*). At −0.5 MPa NaCl, kinetin significantly increased final germination percentage of *A. undulata*, although the effect was minor (<10% increase) and only kinetin and salicylic acid significantly increased germination rate index. At −1 MPa NaCl, *A. undulata* germination was substantially reduced and treatments did not differ significantly. At −1.5 MPa NaCl, no germination was observed in any *Atriplex* species (data not shown).

Seed priming under saline conditions with plant signalling compounds

In *A. amnicola*, GA_3 and salicylic acid significantly increased final germination percentage and germination rate index at 0 and −0.5 MPa compared with water-primed (Fig. 9*a*) or non-primed seed, with GA_3 increasing germination percentage (80%) and germination rate index (70%). Priming seeds with kinetin failed to significantly improve *A. amnicola* germination parameters despite previously identified benefits (Fig. 9*a*). Interaction of GA_3 with either salicylic acid or kinetin failed to further improve germination over the main effect of GA_3. The 3-way interaction of GA_3, salicylic acid, and kinetin significantly improved final germination percentage and germination rate index at −0.5 MPa compared with individual plant signalling treatments, with results comparable with that of germination at 0 MPa (Fig. 9*a*).

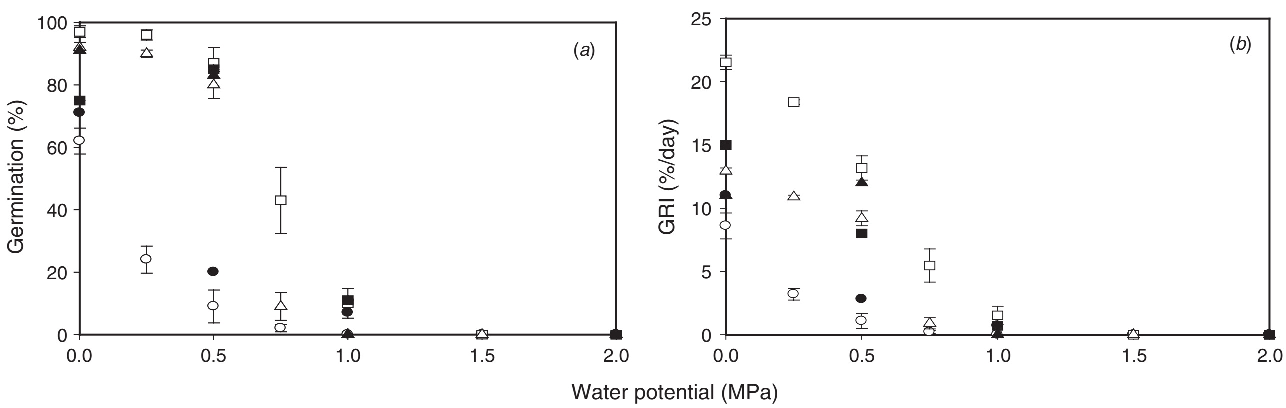

Fig. 7. Effect of different water potentials induced by PEG_{6000} (open symbols) or NaCl (closed symbols) on (*a*) final germination percentage, and (*b*) germination rate index of *A. amnicola* (circles), *A. nummularia* (squares), and *A. undulata* (triangles). Seed germination occurred in the dark at 18/5°C. Values are means ± s.e. ($n = 4$).

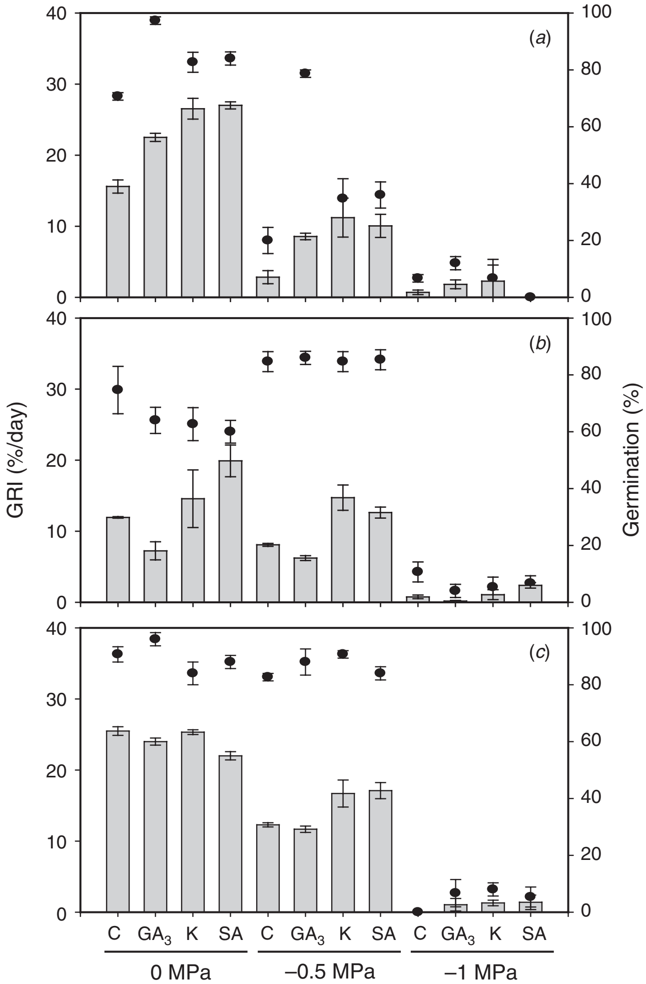

Fig. 8. Effect of plant signalling compounds on germination rate index (bars) and final germination percentage (circles) of (*a*) *A. amnicola*, (*b*) *A. nummularia*, and (*c*) *A. undulata* grown at 0, −0.5, or −1.0 MPa NaCl at 18/5°C in the dark. Plant signalling compounds: C, control (deionised water); GA_3, 1000 ppm GA_3; K, 0.05 mM kinetin; and SA, 0.5 mM salicylic acid. Values are means ± s.e. ($n = 4$).

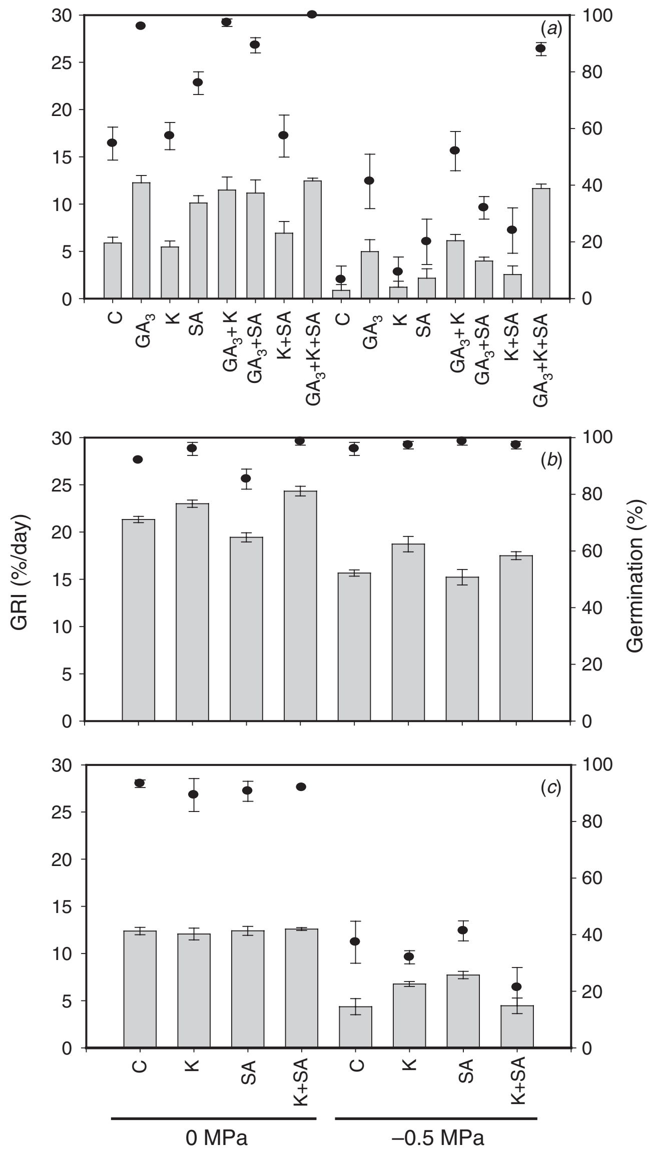

Fig. 9. Effect of priming (*a*) *A. amnicola*, (*b*) *A. nummularia*, and (*c*) *A. undulata* seeds with plant signalling compounds on final germination percentage (circles) or germination rate index (bars) at 0 or −0.5 MPa PEG_{6000}. Plant signalling compounds: H_2O, water; GA_3, 1000 ppm GA_3; K, 0.05 mM kinetin; and SA, 0.5 mM salicylic acid. Values are means ± s.e. ($n = 4$).

Priming seeds of *A. nummularia* in kinetin had no effect on final germination percentage at either 0 or −0.5 MPa (Fig. 9*b*); however, salicylic acid treatment resulted in a slight decrease in final germination percentage and germination rate. Priming seeds in kinetin significantly improved germination rate index under both control and moderate osmotic stress; however, salicylic acid did not significantly increase germination rate index (Fig. 9*b*).

Priming seeds of *A. undulata* in salicylic acid or kinetin had no effect on final germination percentage at either 0 or −0.5 MPa (Fig. 9*c*). Priming seeds with salicylic acid and kinetin increased *A. undulata* germination rate index at −0.5 Mpa, with salicylic acid having the largest effect (Fig. 9*c*). Combining kinetin and salicylic acid in the same priming treatment had no significant effect on final germination percentage or germination rate index (Fig. 9*c*).

Bracteole removal, seed priming, and seedling emergence from saline soils

Intact fruits of *A. amnicola* had the lowest final emergence percentage and emergence rate index at the 2 higher soil salinities, with less than 5% emergence observed (Fig. 10*a*, *b*). Removing the bracteoles of *A. amnicola*

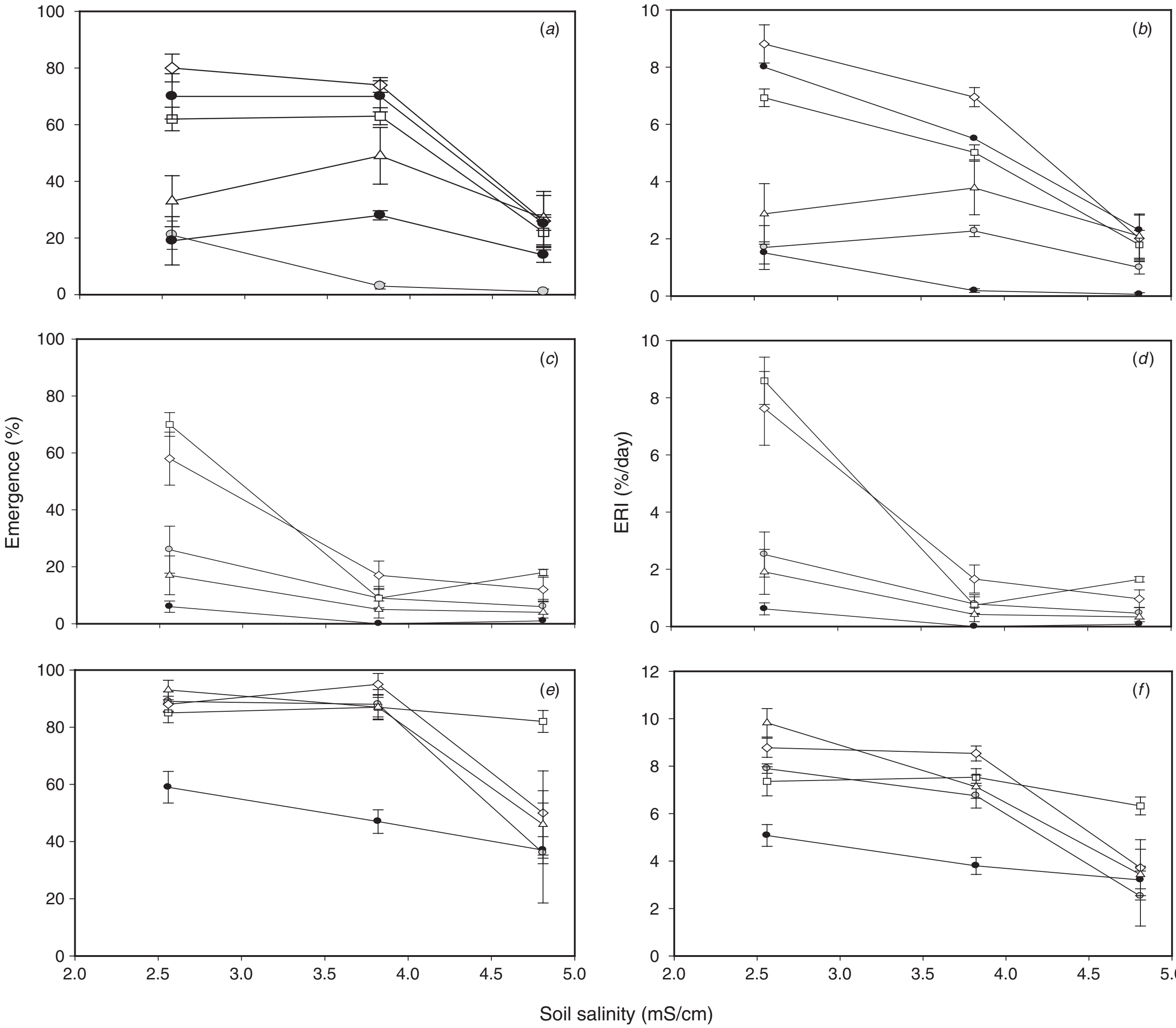

Fig. 10. Effect of bracteole removal and seed priming on final emergence percentage (left column) and emergence rate index (right column) of (*a*, *b*) *A. amnicola*, (*c*, *d*) *A. nummularia*, and (*e*, *f*) *A. undulata* seed at different soil salinities. Treatments were: intact fruit (black circles); bracteole removed, non-primed (grey circle); bracteole removed, water-primed (open triangles); bracteole removed, 1000 ppm GA_3-primed (grey triangles, *A. amnicola* only); bracteole removed, 0.5 mM salicylic acid-primed (diamonds); and bracteole removed, 0.05 mM kinetin-primed (squares). Plants were grown in 23/8°C 12/12 h day/night. Values are means ± s.e. ($n = 4$).

had no effect on emergence parameters at the lowest soil salinity tested ($EC_{1:5}$ 2.56 mS/cm) but significantly increased emergence at higher soil salinities (Fig. 10*a*, *b*). Priming seeds with water significantly improved emergence rates of *A. amnicola* at the 2 lower soil salinities ($EC_{1:5}$ 2.56 and 3.82 mS/cm). Salicylic acid, kinetin, and GA_3 significantly improved emergence of *A. amnicola* at $EC_{1:5}$ 2.56 mS/cm, with salicylic acid having the greatest effect (80% emergence) followed by GA_3 and kinetin (Fig. 10*a*). Effects of plant signalling compounds on values of the emergence rate index reflected final emergence trends (Fig. 10*b*).

Intact fruits of *A. nummularia* had the lowest emergence percentage and emergence rate index at $EC_{1:5}$ 2.56 and 3.82 mS/cm (Fig. 10*c*, *d*). Removing the bracteoles of *A. nummularia* significantly increased emergence at lower soil salinities but had no effect on emergence parameters at the highest soil salinity ($EC_{1:5}$ 4.81 mS/cm) (Fig. 10*c*, *d*). Priming *A. nummularia* seeds after bracteole removal in water had no effect on emergence parameters, whereas priming in salicylic acid or kinetin improved emergence from 5% in the control to 55 or 70%, respectively, at $EC_{1:5}$ 2.56 mS/cm (Fig. 10*c*). At higher soil salinities, salicylic acid and kinetin promoted emergence of

A. nummularia (Fig. 10*c*, *d*). Values of emergence rate index reflected trends observed in *A. nummularia* final emergence percentages (Fig. 10*d*).

Intact fruits of *A. undulata* had the lowest emergence percentage and rates at the 2 lower soil salinities (Fig. 10*e*, *f*). Removing the bracteoles of *A. undulata* significantly increased emergence under lower soil salinities but had no effect on emergence parameters under the highest soil salinity ($EC_{1:5}$ 4.81 mS/cm) (Fig. 10*e*). Priming seeds of *A. undulata* with water, salicylic acid, or kinetin had no effect on emergence percentage under lower soil salinities ($EC_{1:5}$ 2.56 and 3.82 mS/cm); however, kinetin significantly improved emergence percentage under higher soil salinity ($EC_{1:5}$ 4.81 mS/cm) (Fig. 10*e*). Priming seeds with kinetin or salicylic acid significantly increased emergence rate index at $EC_{1:5}$ 3.82 and 4.81 mS/cm, respectively (Fig. 10*f*).

Discussion

This study shows that manipulation of the seed by physical (*A. undulata*) or chemical (*A. amnicola*) means may greatly improve field establishment (1.5- and 4-fold increases, respectively) in saltbush by overcoming asynchronous and unreliable germination (Fig. 10).

Previous reports have placed little importance on understanding the underlying seed dormancy issues and in particular the light requirement of *Atriplex* seeds. Our study shows that seed germination of *Atriplex* species is differentially regulated by the presence of light and spectral composition. *A. amnicola* was found to have a strong light requirement, which is notably different from other species of *Atriplex* including *A. nummularia* and *A. undulata* (Beadle 1952). Exogenous application of 1000 ppm GA_3 was observed to replace the light requirement in *A. amnicola*; this has been observed in other Australian plant families including the Asteraceae (Plummer and Bell 1995). Application of GA_3 can also substitute for cold stratification, which is thought to be an important mechanism responsible for timing of natural halophyte germination (Baskin and Baskin 1998). More importantly, the present study showed that the application of light or GA_3 during the germination/priming phase of *A. amnicola* can improve seed germination rates and final seedling establishment under field conditions. For other *Atriplex* species (i.e. *A. nummularia* and *A. undulata*) where dormancy release is not governed by light, GA_3 application had minimal effect on germination under moderate water stress (-0.5 MPa).

Unlike GA_3, the application of salicylic acid or kinetin had more general effects on *Atriplex* germination under conditions of water deficits (PEG_{6000} or NaCl). Both salicylic acid and kinetin significantly improved germination rate of *A. amnicola*, *A. nummularia*, and *A. undulata* under moderate water stress (-0.5 MPa) while also promoting *A. amnicola* germination under control conditions.

As only subtle differences exist in cellular and metabolic events prior to germination between non-dormant and imbibed dormant seeds (Bewley 1997), the role of salicylic acid and kinetin in improving seed germination across all 3 *Atriplex* species under water/NaCl stress may be a result of interactions with key cellular/metabolic processes controlling germination, including solute leakage, respiration, DNA repair/synthesis, and protein synthesis. Interactions between these signalling compounds and many of the cellular/metabolic processes have previously been identified in plants, and may also correspond to seed. For example, salicylic acid interacts with plant respiration (Bourbouloux *et al*. 1998) and protein synthesis (Jin *et al*. 2000). The mode/site of action of these chemicals during germination currently remains unresolved; however, as both chemicals appear to result in similar germination patterns under stressful conditions, their signalling pathway and interactions require elucidation. Interactions between the chemical signals particularly during the priming phase remain to be determined to identify optimal seed pre-treatments, including the sequence and duration of each treatment.

Differences in *Atriplex* species responses to seed priming were evident in the current study. Seed priming in general had a greater effect on *A. amnicola* germination/emergence compared with *A. nummularia* and *A. undulata*. Priming *A. amnicola* seed with water increased germination at lower salinities, and germination was further stimulated when seeds were primed with plant signalling compounds. *A. nummularia* and *A. undulata* did not respond to priming with water but significant improvements in germination rate were observed with salicylic acid and kinetin priming at various soil salinity levels. Benefits to end-users of species that respond to seed priming are likely to be both direct (faster emergence, more uniform stands, greater stress tolerance) and indirect (lower costs of production), resulting in more sustainable pasture establishment systems.

The role of the bracteoles appeared similar among the species of *Atriplex*. Attached bracteoles of *A. amnicola*, *A. nummularia*, and *A. undulata* reduced both germination rate index and final germination percentage; however, the mere presence of bract material failed to significantly reduce germination parameters. The role of soluble inhibitors in the bracteoles of *Atriplex* species tested in this study and *A. semibaccata* and *A. bunburyana* (data not presented) appears to be negligible. This is in contrast to findings in other *Atriplex* species/populations (Beadle 1952; Campbell and Matthewson 1992). Bracteoles may act as a sink for salts so the difference between our investigation and previous studies may be caused by differences in the maternal environments. There may be benefits for improving seedling establishment by investigating the role of maternal factors, such as soil salinity, water availability at seed maturation (Strawbridge 1995), and build-up of bract inhibitors. Under field conditions, bracteole removal from *A. nummularia* resulted in lower seedling emergence, indicating that bracteoles may have an important role in field establishment of saltbush through interaction with factors not identified in this study.

If seed treatments of any sort are to be used for establishing shrubs on saline soils, the implications of treatments and their relationships with the method of sowing must be understood (Malcolm 1972). The results of this study complement previous research on manipulation of the growing environment and seed placement (Smith and Malcolm 1959), including niche seeding practices (Malcolm *et al*. 1980) and weed control (Vlahos 1997). Our observations resolve the cause of the previously observed inhibitory effect of soil burial on river saltbush where covering intact fruits of *A. amnicola* with 0.002 or 0.005 m of soil reduced emergence by 50 and 95%, respectively (Vlahos 1997). Our observations have important implications for seeding practices of *Atriplex* species as we can now treat seed so that burial can be used to minimise seed desiccation (Sankary and Barbour 1972), improve seedling root establishment, reduce predation stresses, and reduce dark-induced germination suppression. The results from this study also suggest that closely related species do not necessarily behave in the same way, and a cue that unlocks the dormancy mechanism or enhances germination in one species may not necessarily have a similar effect across a whole genus.

Acknowledgments

This research was supported by the Sustainable Grazing of Saline Lands (SGSL) initiative, a subprogram of Land Water and Wool. Land Water and Wool is a R&D funding arrangement between the Australian Wool Innovation Limited and Land and Water Australia. Additional investors include Meat and Livestock Australia, and the CRC for Plant-based Management of Dryland Salinity. The authors also thank Dr David Merritt and Dr Shane Turner for assistance and discussions pertaining to seed dormancy release.

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Manuscript received 1 February 2006, accepted 24 August 2006

Effects of biomass manipulation, seed level modification, site preparation and pasture composition on recruitment of *Austrodanthonia* spp. within existing swards

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Short title: Recruitment of *Austrodanthonia* seedlings

Abstract. Perennial grass content is low in most existing pastures across south-eastern Australia and rehabilitation of these pastures rarely occurs through seedling recruitment. When recruitment happens the rate is less than 1% of the seed bank in most occasions. This paper reports on two field experiments that investigated the effects of biomass manipulation, seed level modification, site preparation and pasture composition on the recruitment of *Austrodanthonia* spp. seedlings. The experiments coincided with drier than average years and although successful recruitment of seedlings occurred in both experiments, survival was extremely low. Control treatments resulted in recruitment of only 1 seedling m^{-2} in Experiment 1 and 0 m^{-2} in Experiment 2 whereas there were 130 m^{-2} and 53 m^{-2} respectively in the best treatments. The recruitment gain from seed addition was 1.3% and 0.5% in Experiment 1 and 2 respectively. Insecticide treatments increased recruitment rates as seed harvesting ants are common in these systems but the benefits were small. The effects of removing competition by herbicide treatment on recruitment were also small. *Austrodanthonia* spp. seedlings were greatest with where bare ground was 35-45%, litter dry matter was 0.5 t ha^{-1} and green dry matter was 0.6 t ha^{-1}. Survival rates

were low through both years and all emerged seedlings in Experiment 2 died by winter. *Austrodanthonia* spp. young plants survival at 24 weeks after emergence was related to 20-35% plant cover. At 52 weeks after emergence when the success of survival was determined, treatment effects in Experiment 1 were not discernible but the number of young plants that survived seemed to have been influenced by the presence of competitive biomass of existing plants.

Additional keywords: seedling recruitment, seedling survival, drought, seed set, plant cover, bare ground

Introduction

Austrodanthonia spp. H. P. Linder are cool season, C3, tufted perennial grasses that remain green throughout the entire year and are amongst the most valuable native grasses in pastoral areas of temperate south-eastern Australia due to their persistence and productivity (Scott and Whalley 1982). In the changing states of grasslands during the early years after European settlement, these short cool season perennial species of *Austrodanthonia* represented disclimax communities in grazing lands of temperate woodlands which were dominated by tall warm season perennial tussock grasses before settlement (Moore 1970).

Austrodanthonia tolerates grazing even at high stocking rates (Lodge 1996). Although these grasses respond to increased fertility (Lodge 1979) they also grow and persist well in unfertilised areas (Lodge 1996), and are highly frost and drought tolerant, although dry matter production and quality is lower than other introduced grasses such as *Phalaris aquatica* L. and *Festuca arundinacea* Schreb. (Robinson and Archer 1988). Selections of *Austrodanthonia* spp. (*e.g.* cultivars Taranna and Bunderra) have been commercialised (Lodge 1996) that are moderately tolerant of low soil fertility and acid soil conditions (Mitchell 2006).

Austrodanthonia spp. seed mostly in spring and shed their dispersal units (caryopsis plus lemma and palea) in December (Hagon 1976). Optimal germination and growth occurs at 20-25°C (Hagon 1976; Hodgkinson and Quinn 1976; Lodge and Whalley 1981; Maze *et al.* 1993; Grice *et al.* 1995; Lodge 2004). The seed of the genus is known to exhibit some dormancy that declines over time (3-18 months) (Hagon 1976; Lodge and Whalley 1981; Maze *et al.* 1993; Grice *et al.* 1995; Lodge 2004). Although germination of *Austrodanthonia* spp. has been well studied (Hagon 1976; Lodge and Whalley 1981; Maze *et al.* 1993; Grice *et al.* 1995; Lodge 2004) few studies have focussed on seedling recruitment in the field (Williams 1970; Williams and Roe 1975; Lodge 1981; Virgona and Bowcher 2000; Lodge 2004). Those studies that have reported successful recruitment have commonly reported low survival rates for the emerged seedlings. Lenz and Facelli (2005) found no evidence of *A. caespitosa* recruitment in the field and suggested that recruitment of native grasses may be naturally rare and more dependent on favourable seasonal conditions especially over the first summer after emergence, a view supported by Lodge (1981). Lenz and Facelli (2005) also suggested recruitment of perennial grasses in the field may be improved if more extreme disturbance (*e.g.* ploughing) or seed addition was used.

Native grasslands often occur on areas that are inaccessible and, or have poor quality soils. Surveys done over recent years (King *et al.* 2006) found *Austrodanthonia* spp. were commonly present on the drier ridges and more acid soils with lower phosphate levels. These grasslands generally have low proportions of perennial grasses and previous research (Michalk *et al.* 2003) indicated that at least 60% perennial grass content is needed to achieve useful impacts on livestock production, weed control, lessen acid soil development and increase water use to minimise salinity problems. In much of the temperate landscape where native grasses are dominant, there is a major problem from invasion by the unpalatable *Nassella trichotoma* (Nees) Hack. ex Arechav. *N. trichotoma* is Australia's worst perennial grass weed, which now occurs over 1 million ha of temperate south-eastern Australia (Jones and Vere 1998) and typically invades over-grazed and degraded native pastures of the temperate perennial pasture zone (Campbell and Vere 1995; Campbell 1998). Many degraded pastures still have a residual of desirable perennial grasses that could be used as the base for seed production to encourage recruitment of new plants, improve perenniality of pastures and reduce weed invasion. *N. trichotoma* seedlings fail to survive when effective competition (>0.5 t DM ha^{-1}) from desirable perennial grasses is maintained over summer after a recruitment event (Badgery *et al.* 2008b).

The aim of the experiments reported in this paper was to better understand the ecological processes that would enhance *Austrodanthonia* spp. recruitment. The experiments reported here investigated methods on how best to deliver the seed to the soil surface (maximising the number of germinable seeds that reach the ground at one time), how the sites at the soil surface can be modified (through disturbance and biomass manipulation) and how to improve *Austrodanthonia* spp. seedlings establishment through the creation of microsites.

Methods

Site

The experiment was located at Trunkey Creek on the Central Tablelands of New South Wales (149°19' E, 33°49' S). The site had a mean elevation of 840 m (a.s.l.) and an average annual rainfall of 800 mm. The site sloped slightly with a north-easterly aspect. Prior to establishment of the experiment the paddock had been rotationally grazed mostly by sheep at a moderate to heavy intensity over for ~150 years. The site had a light brown coloured soil (Oyama and Takehara 1970) with sandy clay loam texture (McDonald and Isbell 1990) and was identified as a Chromosol (Isbell 1996). The experiment was established in August 2006 within an existing *Austrodanthonia* spp. (39 plants m^{-2}) pasture. The dominant species was *Austrodanthonia eriantha* (Lindl.) H.P.Linder but there was a mix of other C3 and C4 perennial grass species interspersed with *N. trichotoma*. Annual grasses were limited to the introduced *Aira* spp. and *Vulpia* spp. and there were limited other weeds. The experiment commenced when the seed heads of *Austrodanthonia* plants were starting to emerge during spring. The site was fenced off throughout the entire study to exclude grazing.

Climate data were measured using data loggers (Tain Electronics®) at the site for the duration of the experiment. Soil temperatures and moisture conditions (gypsum blocks) in the soil surface layer (0-50 mm) were recorded as well as relative humidity, air temperature, solar radiation, wind speed and rainfall. The rainfall and average monthly temperature (minimum and maximum) for the duration of the experiment are summarised in Fig. 1, along with long-term averages (1971-2000). The long-term averages were estimated from the Datadrill® program that predicts climate data from given co-ordinates from surrounding weather stations (Jeffrey *et al.* 2001). Annual rainfall was below average throughout the experiment except in February 2007, June 2007 and February 2008. The mean annual maximum daily temperature was 19.3°C with monthly averages ranging from 27.4°C in January to 10.7°C in July. The mean annual minimum daily temperature was 6.9°C with monthly averages ranging from 13.1°C in January to 0.9°C in July.

[Fig. 1]

Experimental design

The experimental design was a factorial combination of 2 seed delivery mechanism treatments x 3 seed treatments x 3 site preparation treatments laid out in a randomised block with 4 replicates. The experiment was repeated over two years (Experiments 1 and 2); Experiment 2 (second year) being a repeat of Experiment 1 (first year) on an adjoining site, a year later. Plots in Experiment 1 were split into 2 nearby blocks, each with 2 replicates due to local constraints. All plots in Experiment 2 were within a single block of 72 plots.

Treatments

The seed delivery mechanism treatments varied the level of mechanical disturbance, plant cover and structure, and how mature seed was delivered to the soil surface. This phase of treatments was applied when it was considered seeds were reaching maturity but before seed drop. The 2 seed delivery treatments were: uncut (UC or control) and cut and remove (CR). In UC treatment the sward was left uncut with seed heads standing to follow the natural cycle. The CR treatment had herbage mass reduced to a level similar to grazing by cutting to a height of 20-50 mm above ground and the plant matter was removed. This treatment aimed to simulate the physical movement from grazing that would have caused some seeds to drop to the ground, while others would have been consumed through grazing.

The seed treatments were designed to determine if current seed rain would maximise seedling numbers, if it was limiting (or conversely if the number of potential microsites for recruitment were limiting) or if seed predation was a problem. The 3 seed treatments were: no seed addition or control (NS), insecticide application (IS) and seed addition (SA). In IS treatment dead seeds (~50 kg ha^{-1}) treated with Gaucho® (a.i.: 600 g L^{-1} imidacloprid) were added to limit predation from ants. These insecticide treated seeds were tested for germination (no germination recorded) before field application. *P. aquatica* seeds were used as they are very attractive to ants (Campbell 1966; Campbell and Gilmour 1979). SA treatment had extra *Austrodanthonia* spp. seeds (Native Seeds Pty

Ltd, Victoria) added to test if current seed rain had saturated the system and if recruitment and/or microsites were seed limited. Seeds were added as florets, ~440 kg ha^{-1}, which was equivalent to around 50 kg ha^{-1} actual seeds of which ~26 kg ha^{-1} seeds (~3.96 x 10^7 seeds ha^{-1}) were expected to be viable and germinate. The florets were tested for germination (47% at 15°C; 61% at 20°C; 45% at 25°C) before field application. They were not tested for dormancy, though the non-germinating seed probably included dormant forms.

The site preparation treatments were designed to modify the potential sites for seedling recruitment by modifying the soil surface layer and to reduce competition for emerging seedlings. The 3 site preparation treatments included: no preparation or control (NP), herbicide application (HA) and scarify and rake (SR). The plot was left unmodified in NP. In HA a sub-lethal dose of Fusilade® (a.i.: 212 g L^{-1}) at a rate of 250 mL ha^{-1} was applied (6 September 2006 in Experiment 1; 3 September 2007 in Experiment 2) to weaken competition from adult plants and to kill any annual grasses that were germinating prior to *Austrodanthonia* spp. germination events. Since *Vulpia bromoides*, an annual grass, was present at the site, Simazine Flowable Herbicide® (a.i.: 500 g L^{-1}) at a rate of 500 mL ha^{-1} was also applied (29 August 2006 in Experiment 1; 31 August 2007 in Experiment 2). These treatments were applied before any seed addition. In SR treatment the ground surface was lightly scarified using a garden rake, to remove small competitors. Some existing (small) adult plants may have been damaged and, or removed in this process. Approximately 50% of the plot area was scarified uniformly to increase the proportion of bare ground and roughen soil surfaces to increase the potential microsites for seedling recruitment. This treatment aimed to create more microsites that may be favourable for germination under suitable climatic conditions and may have changed the location and density of litter biomass.

Plot layout

Plots were 2 x 2 m. Treatments were applied over the whole 2 x 2 m plot. Each plot had 3 levels of measurement in the layout: 0.9 x 0.9 m, 0.3 x 0.3 m and 0.1 x 0.1 m. The centre of the plot was 0.9 x 0.9 m and permanently marked for routine measurements. Outside of the 0.9 x 0.9 m measurement area was a buffer between the adjoining plots that was used to collect additional measurements (*e.g.* soil seed bank and soil samples). The 0.9 x 0.9 m area was subdivided into nine 0.3 x 0.3 m contiguous quadrats (arranged in a 3 x 3 square) and used to measure biomass and plant species composition. Each 0.3 x 0.3 m quadrat was further divided into 81 0.1 x 0.1 m sub-quadrats arranged within the 0.9 x 0.9 m permanently marked area. Seedling numbers were recorded in these sub-quadrats. The total area for seedling measurement overlaid the same area as the quadrats used for biomass and plant composition estimates. The seedling numbers were combined for the 9 sub-quadrats (0.1 x 0.1 m each) overlaying the 0.3 x 0.3 m quadrat for comparisons at that level.

Measurements

Dry weight ranks of the 3 most abundant species and the total dry matter (DM) of all species were estimated using BOTANAL procedures (Tothill *et al.* 1992). Ranked species were combined into plant functional groups using a subjective method defined by Gitay and Noble (1997) which is based on a combination of life history, physiological and abundance characteristics. Dry weights of standing DM (t ha^{-1}) and litter DM (t ha^{-1}) were estimated separately and the estimates were corrected using 15 to 20 calibration cuts at each sample period (Sanford *et al.* 1998). Sampling for pasture biomass was done every 3 months in the late summer (February), autumn (May), winter (August) and spring (November) of each experimental year. Plant cover, litter cover and bare ground percentages were visually estimated (Sanford *et al.* 1998) and ratings were in 5% increments (*e.g.* 0, 5, 10 to 100). Plant cover was defined as the area on the ground covered by standing biomass when projected vertically on to the soil surface. Litter cover represented the portion of the ground surface covered by detached and dead material excluding the basal area of the standing plants. Bare ground corresponded to the area which was bare in terms of soil exposure plus the area covered by any non-plant material (*e.g.* cow manure, rocks, tree branches) present.

Sampling frequency for seedling monitoring varied with time from germination events. The emergence immediately following treatment application and a subsequent substantial rainfall event was the major germination event to be monitored through the year. Survival was defined as the young plants that lived through the first summer. Initial counts were done within 2-3 weeks of seeds germinating (after treatment application) and then after approximately 6, 24 and 52 weeks. Each sample counted all the seedlings present and marked those newly emerged with coloured nails for the monitoring of young plant survival.

Soil cores were taken across the site to determine the seed bank. The cores were taken at the start of experiment (after treatment application) in both experiments. Samples were only taken in those treatments where natural seed fall was assumed to differ, so the treatments that had addition of extra seeds were avoided. Within the plot, 2 soil cores (0.05 m diameter x 0.05 m deep) were randomly collected - one from each side of the 0.9 x 0.9 m area used for measurements. The cores for each plot were mixed, brought to the glasshouse, sifted, added to the surface of sand filled pots and regularly watered. All seedlings that emerged were identified, counted, recorded and then combined into functional groups. The experiment continued until there were no more seedlings emerging, usually for a period of 2 weeks. An aim was to determine the readily germinable species that could compete with the emerging perennial grass seedlings.

Seed production across the site was estimated through each flowering season, which occurred over the summer. The total number of plants and seed heads in each of those plants were counted in 10 randomly selected 0.3 x 0.3 m quadrats across the site at the time of seed maturity. From these plants 10 seed heads were collected and seeds were counted to estimate the amount of seed per seed head and total seed production.

Austrodanthonia spp. seedlings were first observed in early autumn (March) after summer rainfall received during the months of January and February in each year. The final measurements were made a year later following the first summer after the initial germination event. For Experiment 1 (2007-8) initial observations for seedlings were made on 13 March 2007 followed by young plant survival counts on 19 April 2007, 30 August 2007 and the final count on 12 March 2008. Experiment 2 (2008) followed a similar sequence with seedling counts on 10 March 2008, 21 April 2008 and 28 August 2008. However, for Experiment 2 no observations were taken a year after the recruitment event as due to drought very few seedlings had survived the first six months and that experiment was then terminated.

Analyses

Differences between treatments in total standing biomass, green and litter biomass, each functional plant type biomass, bare ground and plant cover were analysed by Restricted Maximum Likelihood (REML) analysis to account for spatial variability. An irregular grid with a power model and Euclidean distance measure was used due to the arrangement of plots. Significance was determined through Wald tests.

Due to data being non-normal, a Generalised Linear Mixed Model (GLMM) analysis assuming a Poisson distribution with logarithmic link function was used to determine differences in seedling numbers between treatments. Statistical significance was determined through Wald tests.

A General Linear Multiple Regression using a quadratic model (GLM) was used to identify relationships between seedling recruitment and young plant survival with functional plant groups DM (t ha^{-1}), green DM (t ha^{-1}), litter DM (t ha^{-1}), bare ground (%) and plant cover (%). Sequential Bonferroni correction was used for alpha (α) level adjustment to account for multiple comparisons. Cross-correlations in regressions were investigated to avoid confounding of factors. By August 2008 (24 weeks after emergence) there were no surviving young plants in Experiment 2, therefore survival analysis could not be performed and only recruitment analyses are presented. Seedlings data were square root transformed [$\sqrt{(x + 0.5)}$] prior to GLM analysis to ensure homogeneity of variances. All statistical analyses were done using GenStat 9.1® (Payne *et al.* 2006).

Results

Treatments were designed to create variations in the vegetation structure of the sward, modify viable seed levels and generate suitable microsites for germination. Since the subset of treatments in modifying seed levels would not affect the sward structure, the results on grassland description and species composition are presented only for the subset of treatments in seed delivery mechanism and site preparation. Results on seedling recruitment and survival are presented across all subsets of treatments as seed level modification represents one of the most significant factors for emergence of seedlings.

The time of the identified recruitment event after treatment application for Experiment 1 was in March 2007 hence results are presented from late summer 2007 to summer 2008. In Experiment 2 (2008), data for the full year were not collected because there were no surviving seedlings by the end of winter 2008, therefore results for Experiment 2 are presented for the first three seasons of the year until winter (August 2008) when the experiment was terminated. Seedlings recruited in early autumn 2008 did not survive the dry winter therefore final measurements were made in August 2008.

Grassland description

Experiment 1 (2007-8)

At the start of measurements (*i.e.* the time of the identified recruitment event in March 2007) UC had 0.04 ± 0.02 t ha^{-1} more standing dry matter (DM) than CR ($P<0.01$) (Fig. 2-a). Though UC had slightly more standing DM (except in spring 2007) for the rest of Experiment 1, this difference was not statistically significant. There were no significant differences between SR compared to NP, a trend that continued throughout the experiment. No effects of HA on standing DM were observed but HA was designed to kill annual grasses rather than modify vegetation structure. The herbicide was applied about six months before these measurements were taken. There was on average 0.26 ± 0.03 t ha^{-1} of standing DM across all treatments in the beginning (summer 2007) which may be the reason why significant differences were not evident in some of the treatments. Nevertheless, the standing DM across all treatments increased through the year and was highest (1.6 ± 0.1 t ha^{-1}) after 1 year in summer 2008. Most grass growth occurred in spring.

The starting green DM (Fig. 2-b) was so low (0.07 ± 0.01 t ha^{-1}) that no significant difference was observed until the end of the experiment in summer 2008, when CR x HA had the lowest green DM (0.51 ± 0.05 t ha^{-1}) and CR x SR had the highest (0.62 ± 0.05 t ha^{-1}) ($P<0.05$). Green DM was positively correlated with standing DM across all treatments throughout the experiment (*data not presented*). Green DM across all treatments increased during the year reaching 0.57 ± 0.05 t ha^{-1} at the end of Experiment 1 in summer 2008.

When Experiment 1 started (Fig. 2-c), litter DM did not differ between UC and CR but there were difference between NP, HA or SR (P<0.01). HA had the most litter DM (0.36 ± 0.02 t ha^{-1}) and SR had the least (0.29 ± 0.02 t ha^{-1}) compared to NP (0.33 ± 0.02 t ha^{-1}). For the rest of Experiment 1, litter DM remained similar (no significant difference) across all treatments. The general trend across all treatments was a little increase in autumn 2007 (0.34 ± 0.02 t ha^{-1}) before decreasing in winter 2007 (0.09 ± 0.01 t ha^{-1}) and then increasing until the end of Experiment1 in summer 2008. Litter DM across all treatments was less at the end in summer 2008 (0.22 ± 0.01 t ha^{-1}) than at the start in summer 2007 (0.32 ± 0.03 t ha^{-1}).

On average, 46% of the ground surface was made bare in SR in summer 2007 when there was around 12% naturally occurring bare soil surface (Fig. 2-d). More space for germination was available in CR (26 ± 2%) than UC (20 ± 2%) (P<0.001) and in SR (46 ± 2%) than HA (11 ± 2%) or NP (13 ± 2%) (P<0.001); this trend remained the same throughout Experiment 1 except at the end in summer 2008 when UC (11 ± 1%) and CR (12 ± 1%) had the same amount of bare ground. Though bare ground decreased during the year, there was always between 10 and 20% bare ground available across all treatments as potential sites for seedling recruitment.

More plant cover was observed in UC and less in CR at the start of Experiment 1 in summer 2007 (P<0.05) and during winter 2007 (P<0.05) but was the same across all treatments for the rest of Experiment 1 (Fig. 2-e). Plant cover gradually increased after autumn 2007 and was highest in summer 2008 (end) at 45 ± 2% (was 14 ± 1% at the start).

[Fig. 2]

Experiment 2 (2008)

In summer 2008 UC had 0.60 ± 0.02 t ha^{-1} more standing DM than CR (P<0.001) (Fig. 3-a). SR had 0.62 ± 0.03 t ha^{-1} less than NP or 0.67 ± 0.03 t ha^{-1} less than HA (P<0.001), which was in contrast to Experiment 1 when SR did not remove more standing DM because of the low availability of biomass in that year. UC x NP had the highest standing DM (2.4 ± 0.04 t ha^{-1}) (P<0.001) and CR x SR had the lowest (0.92 ± 0.04 t ha^{-1}) (P<0.001), an interaction not observed in Experiment 1. The average standing DM after treatment application in summer 2008 was 1.69 ± 0.04 t ha^{-1}; this was about 7 times that of Experiment 1 but similar to levels at the end of Experiment 1. There was a gradual increase through the seasons across all treatments and standing DM reached 2.8 ± 0.08 t ha^{-1} in the end during winter 2008.

As in Experiment 1 green DM was positively correlated with standing DM across all treatments in Experiment 2 (*data not presented*). In summer 2008 more green DM was present in UC (0.67 ± 0.02 t ha^{-1}) than CR (0.41 ± 0.02 t ha^{-1}) (P<0.001) and less in SR (0.35 ± 0.04 t ha^{-1}) than NP (0.63 ± 0.04 t ha^{-1}) or HA (0.62 ± 0.04 t ha^{-1}) (P<0.001). This trend remained the same throughout the experiment (Fig. 3-b). Similarly, the highest green DM was in UC x NP (0.88 ± 0.03 t ha^{-1}) (P<0.001) and the lowest in CR x SR (0.25 ± 0.03 t ha^{-1}) (P<0.001). Green DM across all treatments increased during the year from 0.5 ± 0.03 t ha^{-1} in summer 2008 to 1.1 ± 0.05 t ha^{-1} in winter 2008. These were higher initial and final values than the year before.

Litter DM was comparatively consistent across all treatments except in the beginning of Experiment 2 (summer 2008) when slightly less litter was present in CR x SR (P<0.001). There was very little litter DM present at the start (0.2 ± 0.02 t ha^{-1}), which declined as the experiment progressed and hardly any litter DM was present in the end of Experiment 2 (0.01 ± 0.001 t ha^{-1}) in winter 2008 (Fig. 3-c). The decline was similar to Experiment 1.

There was around 21% naturally occurring bare ground and SR created on average 52 ± 1% at the start of Experiment 2 in summer 2008 (Fig. 3-d); these were marginally higher proportions than Experiment 1. UC had 7 ± 1% lower bare ground than CR ($P<0.001$) whereas SR had 30 ± 1% higher than NP or HA ($P<0.001$). The most space (52 ± 1%) for germination was available in CR x SR ($P<0.001$) whereas least bare ground (9 ± 1%) was available in UC x NP ($P<0.001$); the trend remained the same throughout Experiment 2. As was the situation in Experiment 1, the percentage of bare ground across all treatments declined through the year and was 12 ± 2% when Experiment 2 ended during winter 2008.

The change in plant cover was similar to Experiment 1 and showed an increasing trend but was comparatively higher (~3 times to Experiment 1) to start with (Fig. 3-e). There was higher plant cover in UC (53 ± 1%) compared to CR (41 ± 1%) ($P<0.001$) and lower cover in SR (31 ± 1%) compared to NP (51 ± 1%) or HA (52 ± 1%) ($P<0.001$). The most cover (61 ± 1%) was present in UC x NP ($P<0.001$) and the least (33 ± 1%) in CR x SR ($P<0.001$); the trend remained the same throughout Experiment 2. The amount of plant cover across all treatments gradually increased from 47 ± 1% in summer 2008 to 72 ± 2% in winter 2008.

[Fig. 3]

Species composition

Even though the site was dominated by *Austrodanthonia* spp., a total of 29 species were recorded during the course of the two experiments. Individual species of *Austrodanthonia* are difficult to distinguish from vegetative structures. The examination of inflorescence and individual florets showed *A. eriantha* was the dominant species at the site. In the central tablelands of NSW, *A. eriantha* along with *A. duttoniana* (Cashmore) H.P.Linder, *A. monticola* (Vickery) H.P.Linder, *A. pilosa* (R.Br.) H.P.Linder, *A. setacea* (R.Br.) H.P.Linder are more common on lower pH soils (Dowling *et al.* 1996a). These species therefore could also be present at the site but at lower proportions as *A. eriantha* was prominently present at the site. *Austrostipa* spp. J. Everett & S.W.L. Jacobs, *Elymus scaber* (R.Br.) Á. Löve and *Microlaena stipoides* (Labill.) R.Br. were the other main perennial grass species. In both experiments *Austrodanthonia* spp. was the dominant plant species hence changes in its DM (Fig. 4) followed similar patterns to the total standing DM (Fig. 2-a, 3-a). For subsequent analyses species were formed into the functional groups: *Austrodanthonia* spp. combined with C3 perennial grasses (AD), C4 perennial grasses (C4), annual grasses (AG) and other species (OTH). The small numbers of forbs, legumes, sedges and broadleaves were combined into a single functional group OTH otherwise there would have been very low and, or missing values limiting the analyses possible.

[Fig. 4]

Experiment 1 (2007-8)

The grassland was dominated by *Austrodanthonia* spp., representing >74% of total DM in all seasons (Fig. 4-a,b). At the start of Experiment 1 in summer 2007 CR did not reduce *Austrodanthonia* spp. DM significantly compared to UC. HA had lower *Austrodanthonia* spp. DM than NP or SR ($P<0.001$) *i.e.* the mild herbicide treatment reduced *Austrodanthonia* spp. biomass. *Austrodanthonia* spp. DM increased across all treatments in Experiment 1 (highest growth rate in spring) from 0.19 ± 0.02 t ha^{-1} in summer 2007 to 1.3 ± 0.1 t ha^{-1} at the conclusion of Experiment 1 in summer 2008. C3 including *Austrostipa* spp., *E. scaber* and *M. stipoides* was the second largest group averaging ~11 to 23% as the experiment progressed. AG was present only during winter (4%). C4 (0.2-0.5%) and OTH (0.7-2.4%) were low throughout Experiment 1. Within each functional group (other than AD) and at each sampling period significant differences were not observed across treatments.

Experiment 2 (2008)

Experiment 2 was located at an adjacent site to Experiment 1. Species composition was similar to Experiment 1 and *Austrodanthonia* spp. dominated species composition in Experiment 2 (Fig. 4-c,d). *Austrodanthonia* spp. accounted for >65% of the total DM and increased from 1.1 ± 0.2 t ha^{-1} in the beginning of Experiment 2 (summer 2008) to 1.8 ± 0.3 t ha^{-1} at the conclusion of Experiment 2 in winter 2008. The proportion of *Austrodanthonia* spp. DM was slightly lower than in Experiment 1 even though the quantity (t ha^{-1}) was significantly higher. During the year there was more *Austrodanthonia* spp. DM in UC than in CR ($P<0.01$), a result not observed in Experiment 1. C3 perennial grasses including *Austrostipa* spp., *E. scaber* and *M. stipoides* was the second largest group averaging ~38% (more than Experiment 1) and was lower in SR than NP or HA ($P<0.05$). AG (5%) was present only during winter and was slightly more in UC than in CR, again a difference not observed in Experiment 1. C4 (0.1-0.5%) and OTH (2-3%) DM production was low and did not differ significantly across treatments at each sampling period, as was the case in Experiment 1.

Recruitment patterns

Flowering and seed set

Inflorescence emergence (initiation of flowering) was observed during October (mid spring) when florets were green and immature. Florets and seeds matured unevenly over a period of several weeks until late December (early summer) when the majority of florets had turned white and fluffy. Maturation usually started at the uppermost tip of the seed head (inflorescence), progressing towards the base over a relatively short period (a few days to a week) turning from green to white and fluffy. The period from flowering maturity (mid spring - early summer) was similar in 2006-7 and 2007-8. Flowering was also noticed in early autumn in 2007 but at a very low scale, and therefore was not monitored as the contribution to the amount of seed set was considered minimal.

Small proportions of florets were harvested at a late stage of floret maturity (early summer) when most florets were white and fluffy, characteristics that indicate when the florets had reached maturity. These collected florets were then used to calculate seed production and undertake germination tests. More seeds were produced in 2007-8 than in 2006-7 (Table 1). Seed weight was 0.64 ± 0.05 mg and germination of florets immediately (1-2 weeks) after collection was 70% at the optimal temperature of 20°C, suggesting that any dormant seed was a minority.

[Table 1]

Seed availability

Perennial grass seed in the soil seed bank was very low compared to annual grasses (AG), broadleaves and legumes in both experiments (Table 1). There were 64 *Austrodanthonia* spp. seeds m^{-2} (0.2%) present in Experiment 1 soil samples (2007) but there was none in Experiment 2 (2008) seed bank. Perennial grass (other than *Austrodanthonia* spp.) seeds were less than or equal to 5% of the total germinating seed bank. More than half the seed bank was comprised of AG in Experiment 1 and by broadleaves in Experiment 2.

Seed set before recruitment was poor in Experiment 1 (14.3 kg ha^{-1}) and slightly better in Experiment 2 (17.1 kg ha^{-1}). Germination rate of freshly fallen seeds was high (67% in Experiment 1; 71% in Experiment 2; Table 1), which reduced the seed available from natural seed set (9.6 kg ha^{-1} in Experiment 1; 12.1 kg ha^{-1} in Experiment 2; Table 2). However, from natural seed fall less than 1% produced a seedling (1 m^{-2}) in Experiment 1 whereas there was none in Experiment 2 across the site (Table 2). Seed addition treatments more than doubled the amount of viable seeds to what was naturally set in each experiment. Through seed addition there was slight improvement in percentage gain, 1.3% in Experiment 1 and 0.53% in Experiment 2 (Table 2). The best treatment without seed addition resulted in 9 seedlings m^{-2} and for the same treatment adding seeds produced 35 seedlings m^{-2} in Experiment 1 (Table 2). In Experiment 2, there was far less recruitment across the site hence the best treatment resulted in only 1 seedlings m^{-2} from natural seeding whereas recruitment of 14 seedlings m^{-2} occurred from seed addition (Table 2).

[Table 2]

General recruitment, overall young plant survival and treatment effects

Experiment 1 (2007-8)

Experiment 1 measurements recorded *Austrodanthonia* spp. seedlings germinating in early March 2007 (Fig. 5-i) after summer rainfall in late February 2007 (68 mm over 11 days; Fig. 1). The greatest seedling number (296 m^{-2}) was observed in UC x SA x SR. *Austrodanthonia* spp. had marginally more seedlings germinated in CR than UC ($P<0.01$) (Fig. 5-i). SA significantly increased seedling numbers compared to NS or IS ($P<0.001$). Higher levels of seedlings were observed in SR in comparison to NP or HA. Within SR, there were more seedlings observed in IS or SA than NS ($P<0.01$) (Fig. 5-iii). Overall, SA x SR had the highest number of established seedlings ($P<0.01$). Almost half the plots (49%) did not have any seedlings emerge. There were four treatments (UC with NS x SR or IS x HA and CR with NS x HA / SR; Fig. 5-iii) that did not recruit any seedlings in any plots. Only 5 plots had more than 100 seedlings m^{-2} and the rest (32 plots) recorded between 1 and 100 seedlings m^{-2}. Seedlings of species other than *Austrodanthonia* germinated in March 2007 across all treatments but in low numbers. The most prominent were AG (~55 seedlings m^{-2}) and broadleaves (~12 m^{-2}). Legumes and perennial grass (other than *Austrodanthonia* spp.) seedlings were minimal (~1-3 m^{-2}). These seedlings did not directly relate to treatments except for AG which occurred more in SR ($P<0.05$).

Substantial loss of seedlings occurred after emergence, although variations across treatments present during germination were evident in mid autumn (6 weeks after emergence) and late winter (24 weeks after emergence) within treatments where young plants survived (Fig. 5-iv,v). On average, only 24 seedlings m^{-2} germinated at the site but there were only 2 seedlings m^{-2} (on average) remaining at the end of week 6 after emergence. However, these seedlings survived throughout the year and there was 1 seedling m^{-2} (on average) still present at the end of first summer. The treatment combination of UC x SA x HA had the highest number of surviving seedlings (19 m^{-2}). Seedlings in all treatments declined at the same rate exponentially from emergence to week 52. At the end in early autumn the following year (52 weeks after emergence) hardly any young plants survived but SR and HA had marginally higher survival rates where young plants were present ($P<0.01$).

[Fig. 5]

Experiment 2 (2008)

Austrodanthonia spp. seedlings were observed in early March 2008 (Fig. 5-ii) after rainfall during February (95 mm; Fig. 1) but in lesser numbers than Experiment 1. On average 10 seedlings m^{-2} emerged across the site. As in Experiment 1, the highest seedling number (112 m^{-2}) was observed in UC x SA x SR. Although substantially less *Austrodanthonia* spp. seedlings emerged in Experiment 2, recruitment of seedlings was slightly more in UC than in CR ($P<0.05$) (Fig. 5-ii) which was in contrast to Experiment 1 where the reverse applied. As in Experiment 1, SA had significantly more seedlings than NS or IS ($P<0.001$), and more seedlings were observed in SR compared to NP or HA ($P<0.001$). There was slight more seedlings in IS than in SA or NS within CR ($P<0.01$) (Fig. 5-iii); this was not observed in Experiment 1. Across all treatments, the most number of seedlings occurred in SA x SR ($P<0.01$), the same effect as in Experiment 1. More than half the plots (54%) did not have any seedlings. Five treatments (UC with NS / IS x HA and CR with NS x HA / SR or IS x NP; Fig. 5-iii) did not recruit any seedlings in any plots. No plots had more than 100 seedlings m^{-2} and the rest (33 plots) recorded between 1 and 100 seedlings m^{-2}. There were a very few seedlings of species other than *Austrodanthonia* in March 2008. Low numbers (2-3 m^{-2}) of other seedlings observed included: AG, broadleaves and legumes. No apparent trend across treatments was observed for these seedlings.

There were no young plants surviving in Experiment 2; all emerged seedlings had died by late winter.

Biophysical factors affecting recruitment

The imposed treatments altered the level of plant cover, bare ground, litter, total, green and functional group biomass. Treatment effects were significant hence the seedling numbers that resulted were likely to reflect the availability of microsites or resource space and the competitive environment created in each treatment. These relationships are examined in this and the next sections in a series of figures and tables. Data are only shown over the range in values of each data subset (per treatment) where a significant relationship was detected. Within each significant relationship for the factor of interest zero values were deleted from the figures for clarity. These relationships are multivariate in nature and in consequence no single factor emerges as dominant in determining seedling numbers. For that reason the individual relationships tend to be variable. In these analyses of components results are presented to investigate physical microsite descriptors (*i.e.* bare ground, litter, plant cover) for their influence on recruitment and competition (*i.e.* functional groups biomass). Within that framework each significant factor is described to identify the limits or quantify the factors where better results in seedling numbers were obtained.

Experiment 1 (2007-8)

Seedling numbers were maximised (~60 m^{-2}) when bare ground was between 10-15% within UC that had SA x NP or HA (Fig. 6-1,2). Presence of litter (~0.5 t ha^{-1}) resulted in the maximum seedling numbers (~15 m^{-2}) in CR that included IS x SR (Fig. 6-5). Green DM was low during seedling emergence but maximum seedling numbers (~290 m^{-2}) were present within UC with SA x SR when green DM was >0.1 t ha^{-1} (Fig. 6-3) or had a lower maximum (~15 m^{-2}) at <0.1 t ha^{-1} green DM in CR that had SA x NP (Fig. 6-6). In CR with IS x HA, less than 0.1 t ha^{-1} *Austrodanthonia* spp. DM maximised (~10 m^{-2}) seedling numbers (Fig. 6-4). In these analyses, bare ground as sites for recruitment was more important than plant cover.

Experiment 2 (2008)

Bare ground of ~35% within UC involving SA x HA, resulted in maximum seedling numbers (~25 m^{-2}) across Experiment 2 (Fig. 6-9); this was the same treatment in Experiment 1 where seedling numbers were related to percent bare ground, but where the maximum seedling numbers (~60 m^{-2}) occurred with less bare ground (10-15%). In CR, seedling numbers were maximised (~120 m^{-2}) when bare ground was 45% or less (Fig. 6-11). Collectively this suggests that bare ground needs to be <50% to maximise seedling numbers; this range was higher than Experiment 1. However, consistent with Experiment 1, bare ground was more important than plant cover in these analyses.

When green DM was 0.6-0.7 t ha^{-1} seedling numbers were maximised (~120 m^{-2}) in UC x SA x SR (Fig. 6-10); the same treatment combination which in Experiment 1 resulted in maximum seedling numbers when green DM was >0.1 t ha^{-1}.

Biophysical factors affecting young plant survival

Experiment 1 (2007-8)

Limited survival of young plants for Experiment 1 after the main recruitment event created difficulties for analysis. The dominant effect of dry conditions overruled other effects associated with microsite characteristics that may have had an influence under normal climatic conditions. Within CR, significant relationships were found with plant cover and green dry matter at 24 weeks after emergence (Table 4). These data indicate that plant cover needed to be between 20-35% for seedlings to survive through the seasonal conditions experienced *i.e.* minimal competition from existing plants was important. The variability in the data resulted in some anomalous results (*e.g.* Fig. 6-8) where the quadratic relationship is probably distorted by the absence of any seedlings surviving in the mid-range of the data set.

Significant relationships were not observed at 52 weeks after emergence.

Experiment 2 (2008)

There were no young plants surviving.

[Table 4]

[Fig. 6]

Discussion

This study aimed to better understand the recruitment and survival mechanism of *Austrodanthonia* spp., one of the most valuable native grasses in pastoral areas of southern Australia. The approach taken was to mimic farm practices that encouraged flowering and seed set (*e.g.* grazing exclusion), to prepare more suitable sites for seedling recruitment (*e.g.* soil disturbance through scarification) and to identify better post-emergence tactics that aided young plant survival in the short to medium-term.

In this study *Austrodanthonia* spp. seedlings were first observed in early autumn (March) in both experiments after significant rainfall events in late summer (Fig. 1). After seed maturation during summer, the rainfall in February (93 mm in 2007; 94 mm in 2008) was the first major precipitation that generated seedlings. Moore (1958) and Hagon (1976) both suggested that cool season species like *Austrodanthonia* would germinate and establish best in autumn and spring. Early autumn observations of *Austrodanthonia* spp. seedlings in this study support these earlier studies, but no seedling recruitment was observed in spring or at any other times of the year, most probably because rainfall was insufficient (Fig. 1) and, or there was low seed availability when other conditions for emergence may have been favourable. In some studies (Harradine and Whalley 1980; Lodge 1981) very few seedlings of native perennial grasses were observed in spring. Therefore, the most reliable time for perennial grass recruitment may be in late summer early autumn following seed set, a view supported by previous observations (Lodge 1981; Dowling *et al.* 1996b). Nevertheless mature seed fall should be followed by a suitable rainfall event as grass seeds germinate and emerge only in the presence of adequate soil moisture (Wilson and Briske 1979; Maze *et al.* 1993; Hamilton *et al.* 1999; Zimmermann *et al.* 2008).

There were very few perennial grass seeds in the soil seed bank compared to annual grasses and broadleaf species (Table 1). Seeds of perennial grasses are usually scarce in the soil, as shown from several other studies (Winkworth 1971; Mott and Andrew 1985; Silcock *et al.* 1990; Bertiller and Coronato 1994; O'Connor 1997; Lodge 2004; King *et al.* 2006). Seed addition caused significantly higher numbers of seedlings than control treatments (Fig. 5-i,ii). Seedlings often did not emerge where no seed was added. The positive effect of seed addition on emergence is in agreement with conclusions from other studies that perennial grasslands are seed limited (Fowler 1986; O'Connor 1996; Hamilton *et al.* 1999; Wilsey and Polley 2003; Zimmermann *et al.* 2008). The dry seasons and low fertility at this site probably resulted in the limited seed set, but whether more seed would be set in better years is not known. In 2000, the site had the nutrients level at 7 mg kg^{-1} P, and 0.2% w/w N (Badgery *et al.* 2008a), which would have further declined over time as the site did not have the history of nutrient addition since then.

Soil scarification did not lead to any significant gain in seedling numbers under natural seeding compared to seed addition in both experiments (Table 2). Scarifying was designed to increase the number of available microsites and reduce plant competition. Lack of seedlings in scarified treatments without seed addition showed that the natural seed set in these dry years was not sufficient to saturate the system. The scenario may improve in better years and future research is needed to assess this as well as other ways of enhancing seed set. Therefore, both seed and microsite availability may have been the prime limiting factors in these experiments. However, it is unlikely that farmers would be able to afford to purchase seed (current prices of some of the native seeds cost as much as \$800-900 kg^{-1}) which means that maximising natural seed set to increase the seed yield and soil scarification before seed set may be their only option to improve the chances of recruitment of native perennial grasses. Recent studies (Kim *et al.* 1990; Hofmann and Isselstein 2004; Liu *et al.* 2008) have also shown that disturbance of the soil surface enhanced emergence and recruitment of perennial grasses.

The insecticide treatment generated only a few more seedlings than the control across the site (Fig. 5-i,ii) and in the best treatment the addition of insecticide generated 9 extra seedlings m^{-2} in Experiment 1 and 3 extra seedlings m^{-2} in Experiment 2. No positive influence was achieved from herbicide application when seed was not added. Perhaps the reason why there were such poor effects of herbicide in these experiments was because the levels of green DM were so low (Fig. 2-b). When translated into the context of a farm the benefits from applying insecticide or herbicide may not be sufficiently important or the costs may outweigh benefits. In practice these treatments would probably only apply in occasional circumstances when problems from ants or weeds were severe. Predation of perennial grasslands seed by ants has been identified as an important factor in limiting recruitment (Campbell 1966; Johns and Greenup 1976; Campbell and Gilmour 1979; Capon and O'Connor 1990; Kerley 1991; Crawley 2000; Kelman *et al.* 2002; Kelt *et al.* 2004; Lenz and Facelli 2005) though it is not clear how big a problem this is for *Austrodanthonia* spp.

Plots with bare ground resulted in more *Austrodanthonia* spp. seedlings emerging in both experiments (Fig. 5-i,ii). This is an understandable result as more bare ground ensures less competition and open spaces for germination, but bare ground could also expose seedlings to drier conditions during the early stages of survival. In these dry years the compromise resulted in more bare ground achieving better results, suggesting that competition from existing plants was the more important constraint on recruitment than seedling exposure. Perennial grasses already possess relatively deep roots and thus are in a position to compete aggressively with young seedlings for the limiting resources of soil moisture and nutrients and, if these two constraints are in sufficient supply, then additionally for light (Cook and Ratcliff 1985; Scott 1997). In field experiments higher seedling numbers were observed when bare ground was 10-15% in Experiment 1 and less than 45% in Experiment 2. There is little evidence that germination is enhanced by vegetation cover (Harper 1977), and most studies on perennial grasses showed negative impacts of established competitors on seedling emergence (Moloney 1990; Aguilera and Lauenroth 1993; Milton and Dean 2000; Zimmermann *et al.* 2008). On the other hand, perennial species that germinate in the open spaces between the bases of perennial tussocks can have higher emergence and survival rates (Lodge 1981) which supports the view that a less dense sward comprising open spaces would improve the chances of seedling emergence.

A low level of litter (~0.5 t ha^{-1}) had a slight positive effect on seedling emergence in this study. Presence of litter is believed to maintain higher soil moisture levels and reduce the rate of drying of soil surface (Evans and Young 1970; McWilliam and Dowling 1970; Mott *et al.* 1976) that would enhance germination (Fowler 1986; Lodge 2004). In both experiments, litter levels were always low, less than 0.5 t ha^{-1} and the effects of high levels of litter could not be established.

In both experiments, green DM was low (<0.3 t ha^{-1} in Experiment 1; <0.9 t ha^{-1} in Experiment 2) when seedlings emerged. This may have resulted in minimal competition to emerging seedlings and might reflect a modification to the sward structure that helped shelter seedlings. The green DM was mostly part of the existing adult perennial plants, rather than from any newly emerging annual plants, which would have competed more with the emerging seedlings. Annual grasses were not prominent in this study but annual grasses are known to compete aggressively with weak perennial grass seedlings in the early stages of recruitment (Lamp *et al.* 2001; Lenz and Facelli 2005). Low levels of annual grass biomass have been shown to have a negative effect on the emergence and survival of *Austrodanthonia* spp. seedlings in glasshouse experiments (Lenz and Facelli 2005). The results show that legumes were minor vegetative components of these pastures which perhaps suggest that the very dry seasons and/or low fertility may have been constraining the growth of legumes. However, the presence of legumes in these pastures and their potential effects in supporting livestock production and in nourishing the companion grasses by supplying a much needed nitrogen input if fertility constraints were alleviated could not be ignored. Given reasonable seasons and sufficient soil phosphorus and sulphur, legumes can be major contributors to pasture production and quality into the future.

Plant cover did not feature in significant relationships at the time of initial seedling emergence, but was more important than bare ground at 24 weeks after emergence in treatments where some young plants survived. Seedlings surviving during late winter were associated with 20-35% plant cover. This result may suggest that through autumn and winter the plant cover helped to shield the seedlings.

While successful recruitment of *Austrodanthonia* spp. seedlings was observed, survival rates of emerged young plants were low. Only a few young plants survived the first summer in Experiment 1 whereas all young plants died by the end of winter in Experiment 2. Recruitment was low in Experiment 2 compared to Experiment 1 (Fig. 5-i,ii). Lodge (1981) identified intraspecific competition from neighbouring mature plants of the same species as the factor responsible for high mortality of native perennial grass seedlings. The limited data obtained on longer term survival suggested that plant competition through the subsequent dry year needed to be significantly reduced to enable any seedlings to survive. It then becomes a judgement as to whether or not this is useful, especially as farmers would probably need to utilise any forage available. It may then be more appropriate to use the forage, especially through the next summer, even though there is some risk that any young plants present may be damaged and then re-employ a strategy to manage recruitment in subsequent years. This would provide a minimal cost solution for farmers, although the rate of grassland rehabilitation would be slow.

The experiments were done in prevailing drought conditions. There was very little follow up rainfall in Experiment 2 (2008) after seedling emergence (Fig. 1) and seedlings may have died before winter rain fell at the site. In Experiment 1 (2007) there was slightly better follow up rainfall after emergence but there was low precipitation in late winter, most of spring and early summer (Fig. 1) and this lack may have drastically reduced the number of young plants at the end of first summer after emergence. Previous studies (Harradine and Whalley 1980; Lodge 1981; Lenz and Facelli 2005) indicate seedling establishment in native pastures is controlled primarily by moisture availability.

This study was done in the region where *N. trichotoma* is prevalent as a major perennial weed that readily invades native pastures. Though the focus was to improve the recruitment of *Austrodanthonia* spp., the limited results obtained in this study provide avenues for future research in investigating methods to encourage recruitment of desirable perennial grasses to 'weed proof' native pastures. Earlier research (Badgery *et al.* 2008b) found that 0.5 t DM ha^{-1} of desirable perennial grasses prevented *N. trichotoma* seedlings surviving through dry summers after a recruitment event. Significant relationships were not observed for surviving *Austrodanthonia* spp. young plants in Experiment 1, but where young plants were still surviving, herbage mass was ~1.5 t ha^{-1}, ranging from 1.0 to 1.8 t ha^{-1}. This may provide a small window of opportunity to foster survival of young *Austrodanthonia* spp. plants as they may be better able to endure slightly more competition than *N. trichotoma* seedlings through dry summers. Future research needs to better refine the level of competition that desirable perennial grass seedlings can manage in this common weed situation.

In conclusion, some success was achieved despite the dry conditions. Recruitment was possible and a small number of seedlings survived indicating some progress was possible even in dry years, with higher recruitment and survival expected in wetter years. The perennial seed bank was low suggesting that current seed set is crucial for recruitment. Seed set was poor in these dry years and seed addition proved successful, but the decision to add seeds is largely impractical in farms due to current high seed costs and the lack of availability of native grass seeds. The practical option is to maximise flowering and seed set through application of summer rest periods timed to suit the phenology of target perennial grass species. Future research needs to investigate ways of increasing seed set in the field. Disturbance of the soil surface improved recruitment possibly through creating microsites. A suitable rainfall event in late summer resulted in successful emergence of seedlings. The initial and subsequent survival conditions were not resolved in this study but limited data indicated that effectively managing competition from existing plants may help survival.

Acknowledgements

The authors would like to thank Rosemary and Michael Ridley for allowing their property to be used for the research. They also thank Helen Nicol for statistical advice.

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Fig. 1 The monthly rainfall, and maximum (Tmax) and minimum (Tmin) temperature at *Austrodanthonia* site plotted with the 30-year average, 1971-2000, from Datadrill® (Jeffrey *et al.* 2001).

Fig. 2 (a) Total standing herbage DM (t ha^{-1}), (b) green herbage DM (t ha^{-1}), (c) litter DM (t ha^{-1}), (d) bare ground, and (e) plant cover percentage over time across UC (uncut), CR (cut & remove), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for Experiment 1; predicted means from Restricted Maximum Likelihood (REML) analysis used; standard error bars presented.

Fig. 3 (a) Total standing herbage DM (t ha^{-1}), (b) green herbage DM (t ha^{-1}), (c) litter DM (t ha^{-1}), (d) bare ground, and (e) plant cover percentage over time across UC (uncut), CR (cut & remove), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for Experiment 2; predicted means from Restricted Maximum Likelihood (REML) analysis used; standard error bars presented.

Fig. 4 The biomass (DM t ha^{-1}) of each functional groups (*Austrodanthonia* spp., annual grasses = AG, C3 perennial grasses = C3, C4 perennial grasses = C4 and others) across UC (uncut), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for (a-b) Experiment 1 and (c-d) Experiment 2; predicted means from Restricted Maximum Likelihood (REML) analysis used.

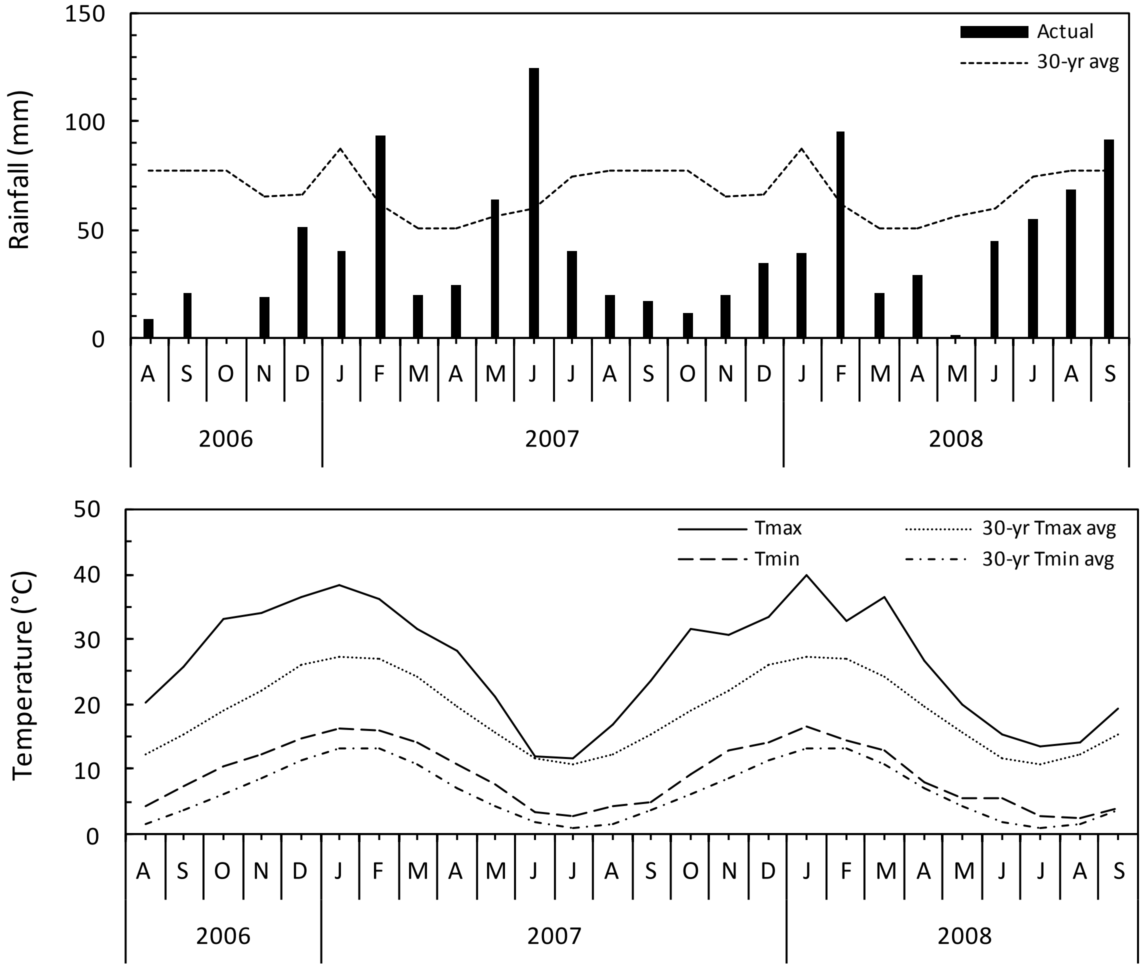
Table 1 Average production of seeds at the *Austrodanthonia* spp. site during the flowering periods (mid spring to early summer) of Experiments 1 and 2 and seedlings (m^{-2}) germinated in the glass house from soil cores (50 mm deep) collected at the start of the experiment in January when seeds were maturing; temperature range used was 20 / 10°C; total numbers (± standard error) in two weeks after sampling.

Table 2 Proportion of recruitment from natural seed set and extra seed addition across the experiment and the best recruitment with or without seed addition in any treatment and in soil scarification treatments.

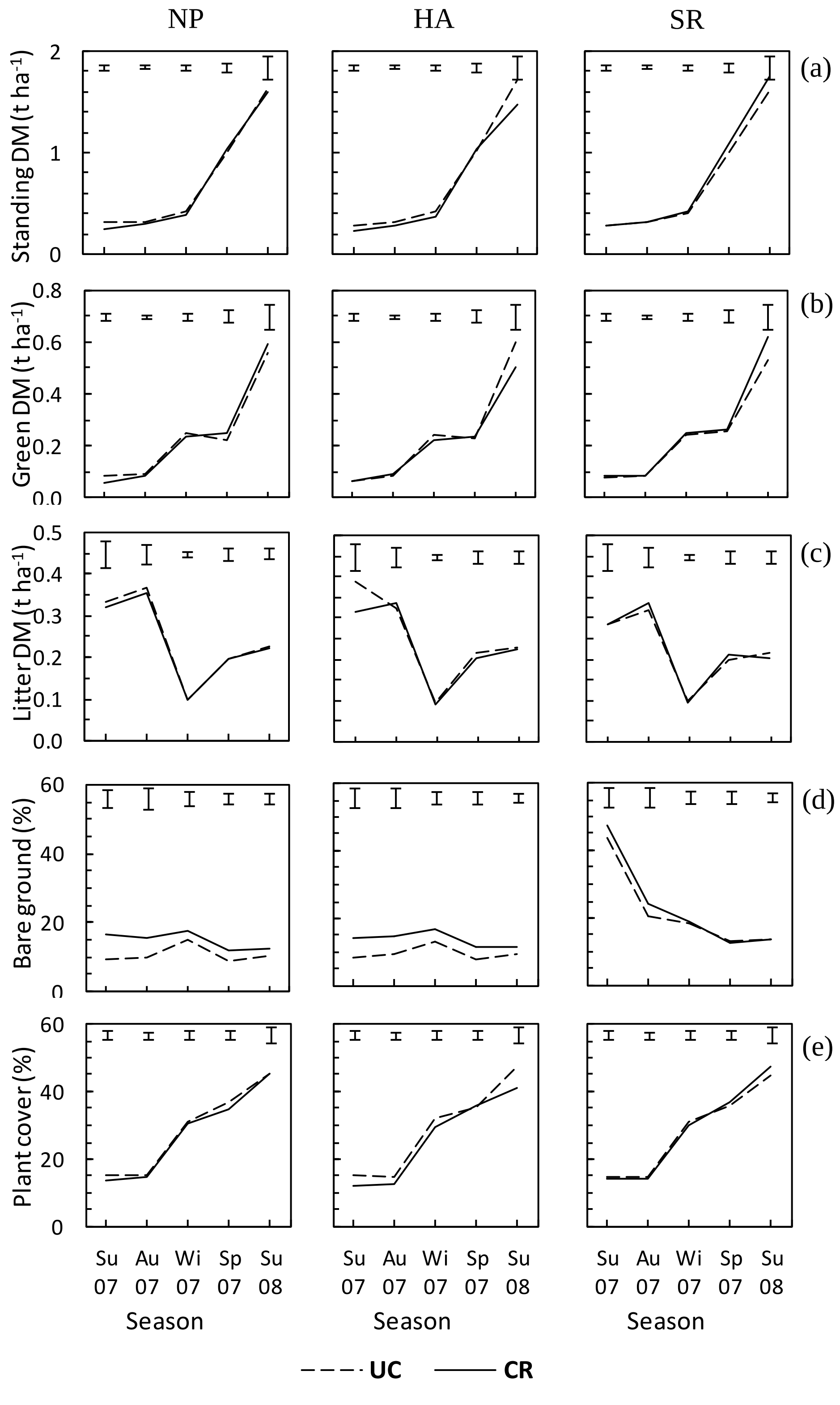
Fig. 5 The average number of *Austrodanthonia* spp. seedlings m^{-2} (logarithmic scale, n + 1) at emergence (March) across broad treatment groups of UC (uncut), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments for (i) Experiment 1 (2007-8) and (ii) Experiment 2 (2008), and across all treatment combinations at (iii) emergence (March), (iv) 6 weeks after emergence (April), and (v) 24 weeks after emergence; back-transformed means from Generalised Linear Mixed Model (GLMM) analysis used; for graphs (i)-(ii), within each subset of treatments in the same year, and for graphs (iii)-(v), within the same year, columns with the same letter are not significantly different, $P<0.05$.

Table 3 General Linear Multiple Regression (quadratic model) equations predicting seedling numbers across UC (uncut), CR (cut & remove), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments with significant relationships; where SN = √ (seedling number m^{-2} + 0.5); AD = *Austrodanthonia* spp. DM t ha^{-1}; OTH = other functional group DM t ha^{-1}; G = green DM t ha^{-1}; L = litter DM t ha^{-1}; B = bare ground %, C3 = C3 functional group DM t ha^{-1}; PC = plant cover %, for *Austrodanthonia* spp. seedlings; only treatment combinations with significant model presented; the most significant factor was regressed against the number of seedlings (transformed) in Fig 6.

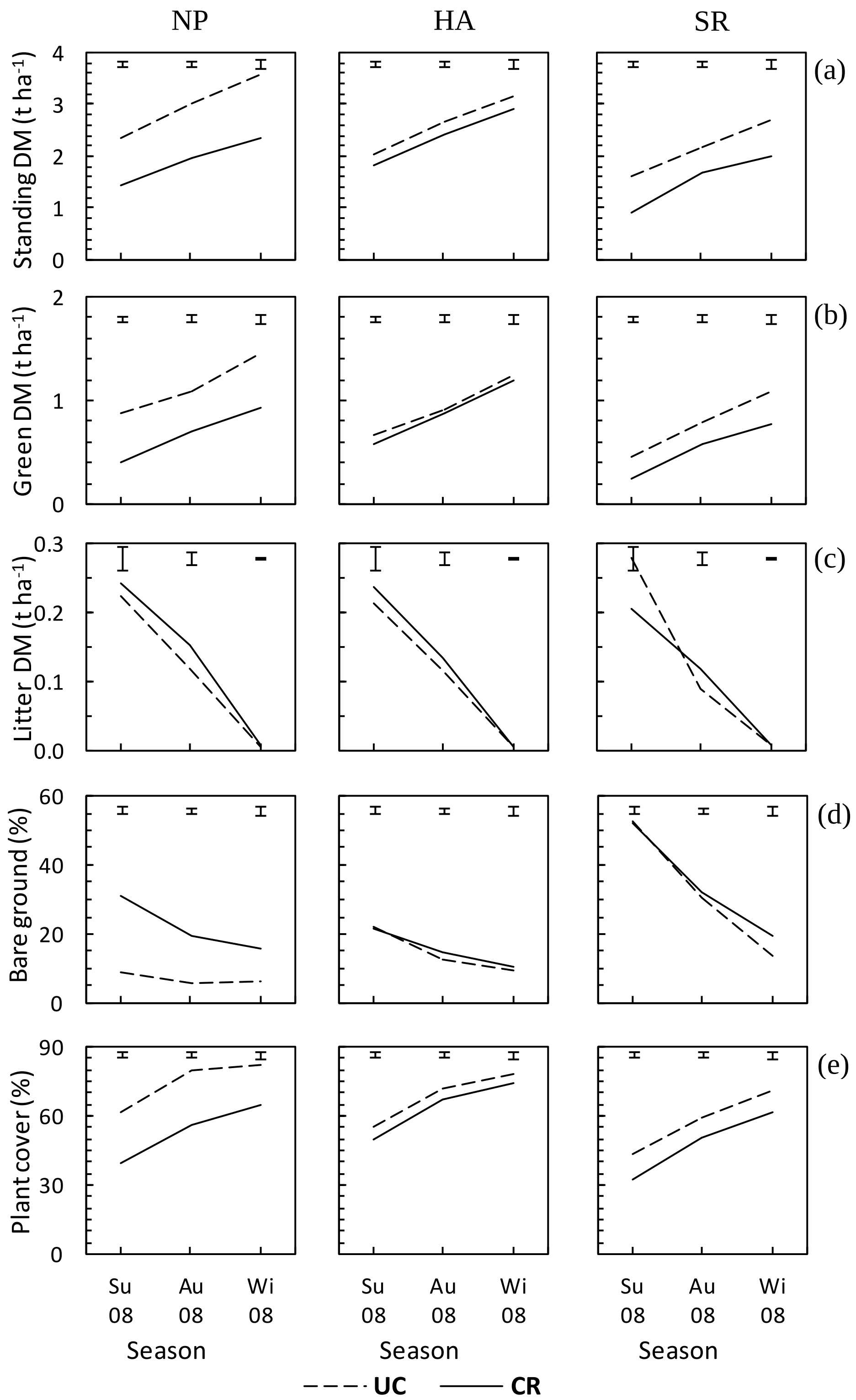
Fig. 6 *Austrodanthonia* spp. seedlings m^{-2} (√ (n + 0.5) transformed) compared to significant factors of General Linear Multiple Regression in Table 3; the solid line represents the most significant factor in the regression model; weak quadratic relationship not shown in graphs 4, 6, 9 and 10; equation numbers from Table 4 correspond to individual graphs.


Rainfall (mm)
150
100
50
0
Actual
30-yr avg
A S O N D J F M A M J J A S O N D J F M A M J J A S
2006
2007
2008
Temperature (°C)
50
40
30
20
10
0
Tmax
Tmin
30-yr Tmax avg
30-yr Tmin avg
A S O N D J F M A M J J A S O N D J F M A M J J A S
2006
2007
2008

1 Fig. 1

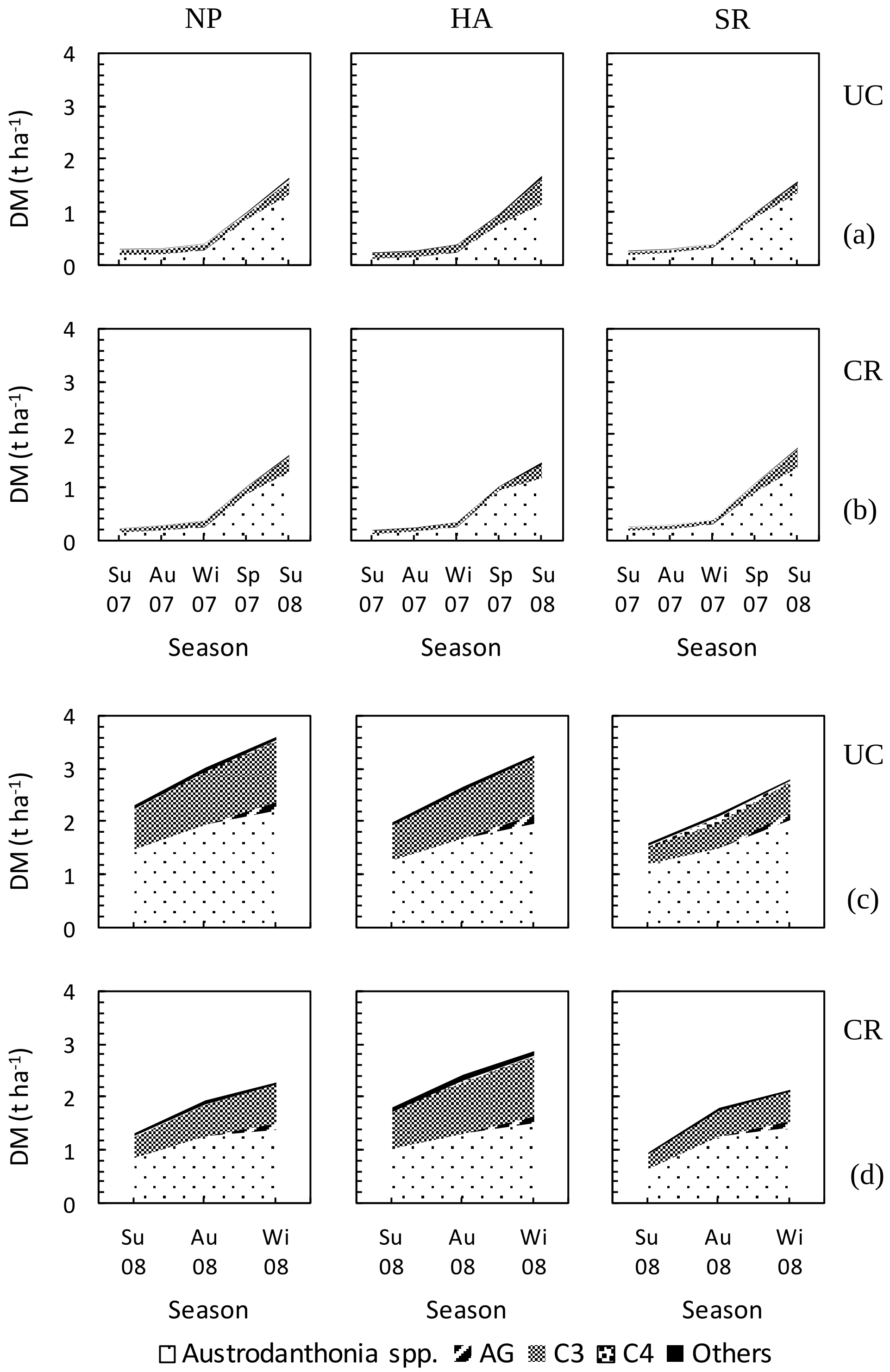

NP
HA
SR
Standing DM (t ha-1)
Green DM (t ha-1)
Litter DM (t ha-1)
Bare ground (%)
Plant cover (%)
(a)
(b)
(c)
(d)
(e)
Su 07
Au 07
Wi 07
Sp 07
Su 08
Season
UC
CR

1 Fig. 2



NP
HA
SR
Standing DM (t ha-1)
Green DM (t ha-1)
Litter DM (t ha-1)
Bare ground (%)
Plant cover (%)
(a)
(b)
(c)
(d)
(e)
Su 08
Au 08
Wi 08
Season
UC
CR

1 Fig. 3


NP
HA
SR
UC
CR
UC
CR
(a)
(b)
(c)
(d)
DM (t ha-1)
0
1
2
3
4
Su 07
Au 07
Wi 07
Sp 07
Su 08
Su 08
Au 08
Wi 08
Season
Austrodanthonia spp.
AG
C3
C4
Others

1 Fig. 4

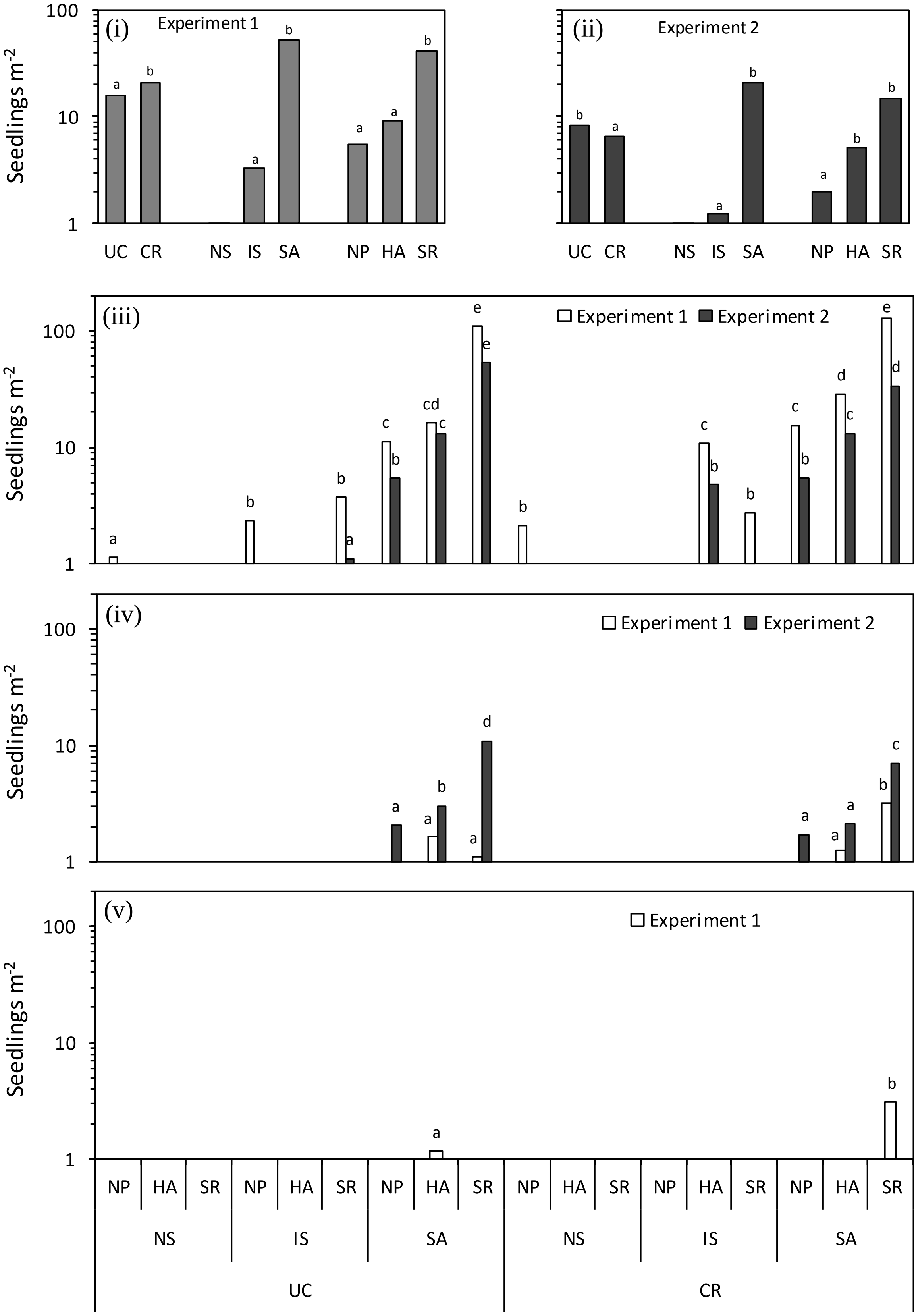
Table 1

	2007-8 (Expt 1)	2008 (Expt 2)
Seeds m^{-2}	2236	2678
Seed yield kg ha^{-1}	14.3	17.1
Germination (% at 20°C)	67	71
seedlings m^{-2}		
Austrodanthonia spp.	32 ± 22	0
Perennial grasses	366 ± 214	541 ± 204
Annual grasses	9581 ± 2400	3629 ± 659
Broadleaves	4488 ± 563	5793 ± 406
Legumes	2371 ± 1280	524 ± 165

Table 2

Seed			Recruitment		Best recruitment (m^{-2})	
mode	amount		m^{-2}	%	any treatment	soil scarification
Experiment 1 (2007-8)						
Natural	1498 m^{-2}	9.6 kg ha^{-1}	1	0.06	9	0
Added	3959 m^{-2}	25.5 kg ha^{-1}	52	1.3	35	296
Experiment 2 (2008)						
Natural	1901 m^{-2}	12.1 kg ha^{-1}	0	0	1	2
Added	3959 m^{-2}	25.5 kg ha^{-1}	21	0.53	14	112

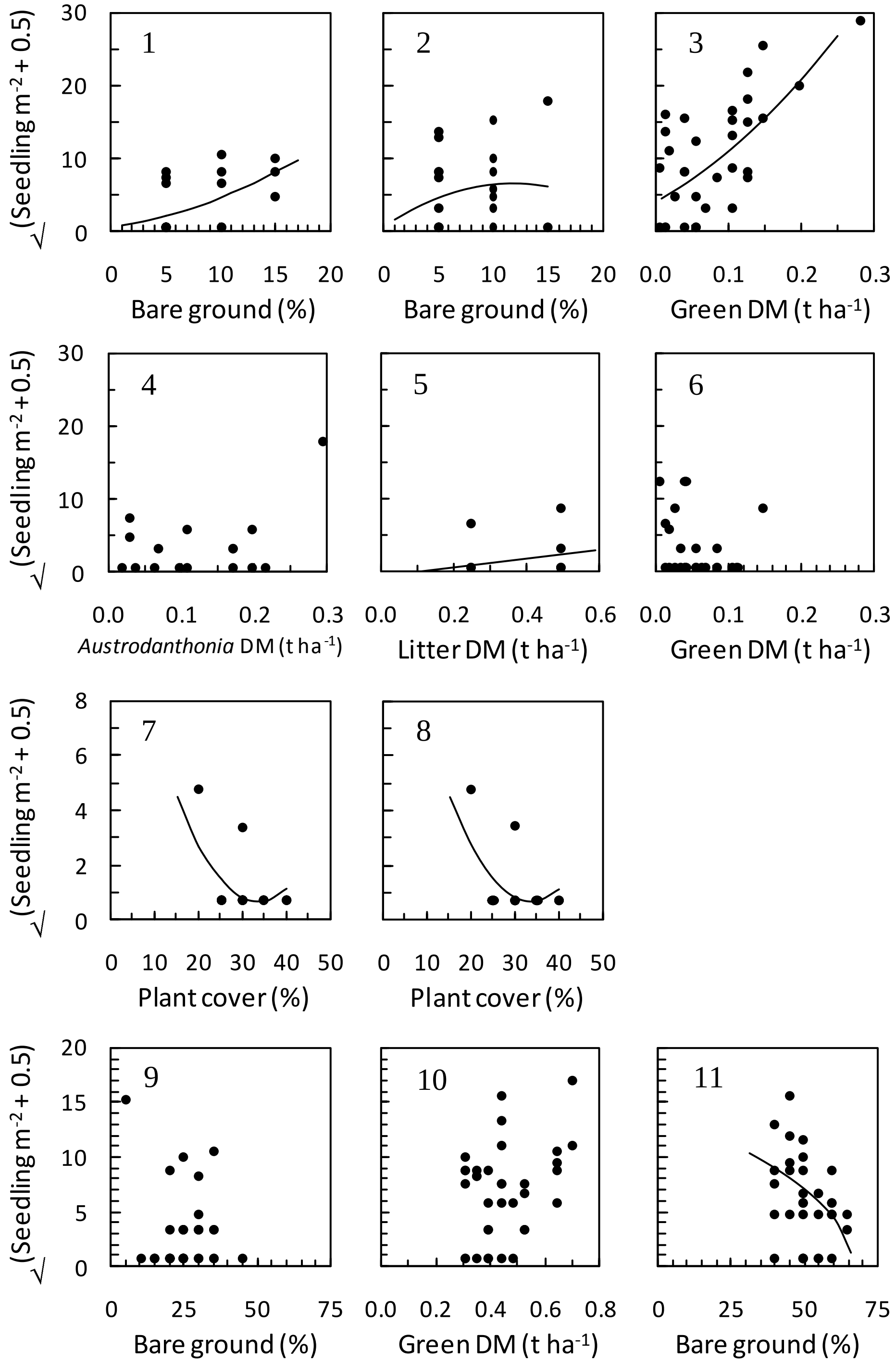
Note: The amounts of seed in column 3 were calculated from the data in Table 1 after accounting for germination percentages and the recruitment percentages in column 5 for the seed added treatments were calculated as the recruitment (natural - added) of the total added seed.


(i)
Experiment 1
(ii)
Experiment 2
Seedlings m-2
UC CR NS IS SA NP HA SR
(iii)
(iv)
Experiment 1
Experiment 2
(v)
NP HA SR
NS IS SA
UC CR

1 Fig. 5

Table 3

Treatment			No	Equation	Adj R^2	P-value
At emergence (Experiment 1: March 2007)						
UC	SA	NP	1	$SN = 0.83 + 0.17B + 0.021B^2$	0.37	< 0.001
		HA	2	$SN = 0.79 + 0.97B - 0.041B^2$	0.18	0.01
		SR	3	$SN = 4.08 + 54.1G + 146G^2$	0.48	< 0.001
CR	IS	HA	4	$SN = 6.89 - 112.4AD + 418.4AD^2$	0.32	< 0.001
		SR	5	$SN = - 0.7 + 6.43L$	0.10	0.03
	SA	NP	6	$SN = 7 - 174.4G + 1205G^2$	0.20	0.009
Overall				$SN = 1.16 + 13.1OTH + 38.4OTH^2 + 0.083B - 0.00087B^2$	0.06	< 0.001
At 24 weeks after emergence (Experiment 1: August 2007)						
CR	IS	HA	7	$SN = 13.11 - 0.74PC + 0.011PC^2$	0.24	0.004
	SA	SR	8	$SN = 12.14 - 0.83PC + 0.015PC^2$	0.15	0.03
Overall				$SN = 1.59 - 4.02G + 4.6G^2$	0.01	0.013
At emergence (Experiment 2: March 2008)						
UC	SA	HA	9	$SN = 168.2 - 11.5B + 0.203B^2$	0.2	0.01
		SR	10	$SN = 370 - 1541G + 1804G^2$	0.2	0.01
CR	SA	SR	11	$SN = 199 - 3B$	0.13	0.01
Overall				$SN = -2.28 + 0.14B + 0.0026B^2$	0.03	< 0.001



√(Seedling m-2 + 0.5)
1
2
3
Bare ground (%)
Bare ground (%)
Green DM (t ha-1)
4
5
6
Austrodanthonia DM (t ha-1)
Litter DM (t ha-1)
Green DM (t ha-1)
7
8
Plant cover (%)
Plant cover (%)
9
10
11
Bare ground (%)
Green DM (t ha-1)
Bare ground (%)

1 Fig. 6

Effects of grazing, pasture cropping, seed level modification, herbicide application and pasture composition on recruitment of *Bothriochloa macra* within existing swards

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Short title: Recruitment of *Bothriochloa macra* seedlings

Abstract. Many existing pastures in south-eastern Australia have low perennial grass content and limited research has been done to investigate low-cost means of encouraging the recruitment of new desirable perennial grasses in these existing pastures. This paper reports on a field experiment that investigated the effects of grazing, pasture cropping, seed level modification, herbicide application and pasture composition on the recruitment of *Bothriochloa macra* seedlings. Successful recruitment of *B. macra* was observed but survival rates were low through the year Recruitment rate proportional to total seed set was only 0.5% and the control treatment had no recruitment compared with 73 seedlings m^{-2} in the best treatment which was pasture cropped, seed added and herbicide applied. The recruitment gain from seed addition was 1.9% which indicated seed was less limiting for this species and availability of microsites may be a major constraint to recruitment as scarification through pasture cropping substantially increased seedling numbers (279 m^{-2}). The effects of herbicide treatment on recruitment were small. Recruitment was higher with 35-55% bare ground and less than 2.2 t ha^{-1} litter DM. *B. macra* young plants survival at 24 weeks after emergence was related to 20-35% plant cover. At 52 weeks after emergence

when the success of survival was determined, treatment effects were not discernible but the number of young plants that survived seemed to have been influenced by the presence of competitive biomass of existing plants.

Additional keywords: seedling recruitment, seedling survival, grazing, pasture cropping, seed set, pasture composition

Introduction

Bothriochloa macra (Steud.) S.T. Blake is a slender, tufted, warm season, C4, native perennial grass which often dominates over-grazed natural pastures and frequently colonises disturbed areas but disappears from fertilised pastures with introduced species (Lamp *et al.* 2001; Waters *et al.* 2001) in temperate Australia. Following the demise of other more palatable perennial grasses through heavy grazing, *B. macra* invades areas from which surface soil has eroded and frequently becomes dominant from northern to central New South Wales (NSW) (Moore 1970). Leaves are usually a reddish or purplish colour and the proportion of leaves to stems and seed heads is low (Robinson and Archer 1988). Stems are of low acceptability and digestibility to livestock, but the high quality leaves are readily eaten when green (Mitchell 2006). Despite being generally regarded by farmers as a less palatable species, *B. macra* is actually similar in its protein content to or better than many other common C4 species such as *Panicum coloratum* L., *Chloris gayana* Kunth and *Astrebla* spp. (Waters *et al.* 2001). However the species is inadequate in supporting highly productive livestock enterprises due to low herbage production in winter and its inability to provide green digestible leaf during winter when the needs of reproductive livestock are at the greatest (Garden *et al.* 2005).

B. macra is highly persistent and productive during droughts and valuable for soil conservation purposes in waterways and heavily grazed summer pastures (Cunningham *et al.* 1981; Lamp *et al.* 2001; Waters *et al.* 2001). It occurs on a variety of soil types in humid areas but is restricted to run-on areas, including clay soils, in drier areas. Although widespread, *B. macra* is not found on more acid soil types. *B. macra* has been reported to be responsive to fertiliser (Cook *et al.* 1976) but it grows and persists as well as many alternative species in less-fertile areas (Mitchell 2006). The species responds positively to grazing (Mitchell 2006).

B. macra flowers mainly in early summer and most dispersal units (caryopsis plus lemma and palea) are shed in late summer to early autumn (Hagon 1976; García-Guzmán *et al.* 1996). Seeds germinate between 20 and 40°C, with optimal germination at 25-30°C (Hagon 1976; Lodge and Whalley 1981; Maze *et al.* 1993; Grice *et al.* 1995). The species is known to exhibit some dormancy, which when subjected to light and the passage of time (4-6 months) dissipates (Hagon 1976; Lodge and Whalley 1981; Maze *et al.* 1993). However, Lodge (1981) reported freshly fallen seeds germinated readily when temperature and rainfall conditions were suitable which implies that seed dormancy in *B. macra* may not limit germination during and immediately after seed fall provided conditions are favourable for germination.

A number of germination studies have been done for *B. macra* (Hagon 1976; Lodge and Whalley 1981; Maze *et al.* 1993; Grice *et al.* 1995) but comparatively less research has investigated the recruitment processes of *B. macra* within existing swards. Lodge (1981) studied emergence and survival of seedlings of warm-season native perennial grasses (including *B. macra*) in both native pastures and sown monospecific plots on the northwest slopes of NSW and concluded that the most favourable period for the successful emergence and establishment of warm-season grasses was from mid-summer to early autumn. However, little recruitment was observed for any of the native grasses studied

(Lodge 1981). The observation of minimal recruitment in native grasses (including *B. macra* and *Austrodanthonia eriantha* Lindl.) was further noted by Semple *et al*. (1997).

In recent years, as farmers have sought more sustainable, lower-cost farming systems, new technologies have emerged such as pasture cropping, a form of relay intercropping where two or more crops are grown simultaneously for part of their life cycle (Andrews and Kassam 1976; Vandermeer 1989). These practices are designed to improve the productivity of perennial grass pastures. As practiced in New South Wales, pasture cropping is a technique where winter growing cereal crops (*e.g.* wheat) are sown into existing perennial grass swards of C4 grasses using sod seeding equipment that has minimal impact on the dominant perennial plants. Due to differences in growth patterns, competition from existing perennial grasses has minimal effects on the cereal crop. In turn, the reduction in annual grasses due to competition from the winter cereal and the soil disturbance within drill rows create suitable conditions for native grass recruitment in the following summer (Seis 2004). This cropping-grazing system has been developed by innovative famers in central NSW. The aims of the approach are to minimise costs in growing the winter crop, produce both forage and grain in the same paddock at different times of the year, and to optimise the rehabilitation of the perennial grass sward. There is considerable interest in how to optimise recruitment within such a system. Anecdotal evidence suggests this technique stimulated perennial grass seedlings to increase in number and diversity (Seis 2004). *B. macra* is a common species in the areas where these techniques are being adopted by farmers.

The aim of the experiment reported in this paper was to understand the mechanisms of *B. macra* recruitment within existing swards and identify what practices best foster seed production (through grazing exclusion), deliver the seed to the soil surface and modify the soil surface (through pasture cropping and grazing) to create microsites to improve the chances that seeds will successfully establish.

Methods

Site

The experiment was located at the Wellington Research Services Centre on the Central Slopes of New South Wales (148°58' E, 32°30' S) within a larger experiment designed to investigate the impacts of pasture cropping on the production and dynamics of existing pastures (Millar and Badgery 2009). The site had an elevation of 300 m (a.s.l.) and an average annual rainfall of 618 mm. There was a slight easterly slope on the site. Over the past 30 years, the site had been very occasionally cropped and fertilised. The site had a red soil (Oyama and Takehara 1970) with loam texture (McDonald and Isbell 1990) and was classified as a Dermosol (Isbell 1996) with pH 6.2. The experiment was established in autumn 2006 within the existing *B. macra* dominant pasture (33 plants m^{-2}) and continued until late summer 2008. The site contained significant proportions of annual grasses, legumes and broadleaf weeds.

Climate data measured at the site included temperature, soil moisture (0-50 mm), relative humidity, air temperature, solar radiation and wind speed using data loggers (Tain Electronics®) for the duration of the experiment. Rainfall figures were obtained from the weather station located <0.5 km from the experimental site. The rainfall and average monthly temperature (minimum and maximum) for the duration of the experiment are summarised in Fig. 1, along with the long-term averages (1946-2005) obtained from the official website of Australian Government Bureau of Meteorology (BOM 2008). The BOM weather station had been at the same location as the weather station used for this experiment, before it was decommissioned in 2004. Rainfall was below the long-term average in most months during the experiment. The mean annual maximum daily temperature was 22.8°C with monthly averages ranging from 31.2°C in January to 14.1°C in July. The mean annual minimum daily temperature was 10.5°C with monthly averages ranging from 17.5°C in January to 3.4°C in July. Average monthly temperatures (minimum and maximum) were a few degrees higher than the long-term averages in most months during the experiment.

[Fig. 1]

Experimental design

The experiment was based upon a split-plot design of ungrazed, grazed and pasture cropping treatments combined factorially with seed and herbicide treatments, laid out in a randomised block with 4 replicates. The field experiments were planned to be done over two years to capture establishment of *B. macra* seedlings in two different years but only one year was possible due to the unavailability of the site from the second half of 2008 due to a change in plans by the owner.

Treatments

Treatments were designed to generate variations in the amount of plant competition, cover / shading, litter, bare ground and surface soil moisture levels, factors known to influence seedling recruitment *in situ* (Dowling *et al.* 1971; Wilson and Briske 1979; Fowler 1986b; Moloney 1990; Lauenroth *et al.* 1994; Milton and Dean 2000; Lodge 2004; Zimmermann *et al.* 2008). The treatments were based on a range of practices that could be readily used on farms to enhance perennial grass recruitment.

Fenced enclosures were used to separate ungrazed or control (UG) from grazed (GR) main plots. Pasture cropped (PC) plots had been established in 2006 (Millar and Badgery 2009). The cereal crop used was *Triticum aestivum* L. (wheat, variety Ventura). In GR treatment the plots were grazed prior to sowing in May-June at the same time as in the larger pasture cropping study (Millar and Badgery 2009). As 2006 became an extremely dry year, crops were utilised for grazing instead of being harvested for grain. A second grazing occurred in November-December period. Grazing would cause some perennial grass seeds to drop to the ground, while others may be consumed and hence alters the amount of seed available at one time. For PC treatment a smaller area within the pasture cropping treatment of the larger experiment that had been cropped in 2006 was used. It was anticipated that disturbance from the PC treatment to the soil surface would increase the potential microsites for seedling recruitment through the availability of more bare ground and rougher soil surfaces.

The seed treatments were designed to determine if current seed rain was adequate. The 2 seed treatments were: no seed addition or control (NS) and seed addition (SA). In SA treatment extra *B. macra* seeds were added on 30 August 2006 to test if current seed rain had saturated the system, if recruitment was seed limited and, or if more suitable microsites exist where seedlings can establish than current seed set could saturate *i.e.* whether or not the available microsites were limiting. The aim was to flood the system with additional seeds. Seeds were added as florets, ~160 kg ha^{-1}, which was equivalent to around 50 kg ha^{-1} actual seeds of which ~23 kg ha^{-1} seeds (~2.20 x 10^{7} seeds ha^{-1}) were expected to be viable and germinate. The florets were tested for germination (44% at 25°C; 47% at 30°C; 39% at 35°C) before applying in the field.

Reducing competition treatments were designed to reduce competition for emerging seedlings through herbicide application. The 2 treatments were no herbicide application or control (NH) and herbicide application (HA). In HA treatment a sub-lethal dose of Fusilade® (a.i.: 212 g L^{-1} FLUAZIFOP-P present as the butyl ester) at a rate of 250 mL ha^{-1} was applied (11 August 2006) to weaken competition from adult plants and to kill any annual grasses that were germinating prior to *B. macra* germination events. This treatment was applied before any seed addition.

Plot layout

Plots were 2 x 2 m. Treatments were applied over the whole 2 x 2 m plot. Each plot had 3 levels of measurement in the layout: 0.9 x 0.9 m, 0.3 x 0.3 m and 0.1 x 0.1 m. The centre of the plot was 0.9 x 0.9 m and permanently marked for routine measurements. Outside of the 0.9 x 0.9 m measurement area was a buffer between the adjoining plots that was used to collect additional measurements (*e.g.* soil seed bank and soil samples). The 0.9 x 0.9 m area was subdivided into nine 0.3 x 0.3 m contiguous quadrats (arranged in a 3 x 3 square) and used to measure biomass and plant species composition. Each 0.3 x 0.3 m quadrat was further divided into 81 0.1 x 0.1 m sub-quadrats arranged within the 0.9 x 0.9 m permanently marked area. Seedling numbers were recorded in these sub-quadrats. The total area for seedling measurement overlaid the same area as the quadrats used for biomass and plant composition estimates. The seedling numbers were combined for the 9 sub-quadrats (0.1 x 0.1 m each) overlaying the 0.3 x 0.3 m quadrat for comparisons at that level.

Measurements

Dry weight ranks of the 3 most abundant species and the total dry matter (DM) of all species were estimated using BOTANAL procedures (Tothill *et al.* 1992). Ranked species were combined into plant functional groups using a subjective method defined by (Gitay and Noble 1997) which is based on a combination of life history, physiological and abundance characteristics. Dry weights of standing DM (t ha^{-1}) and litter DM (t ha^{-1}) were estimated separately and the estimates were corrected using 15 to 20 calibration cuts at each sample period (Sanford *et al.* 1998). Sampling for pasture biomass was done every 3 months in late summer (February), autumn (May), winter (August) and spring (November) of each experimental year. Plant cover, litter cover and bare ground percentages were visually estimated (Sanford *et al.* 1998) and ratings were in 5% increments (*e.g.* 0, 5, 10 to 100). Plant cover was defined as the area on the ground covered by standing biomass when projected vertically on to the soil surface. Litter cover represented the portion of the ground surface covered by detached and dead material excluding the basal area of the standing plants. Bare ground corresponded to the area which was bare in terms of soil exposure plus the area covered by any non-plant material (*e.g.* cow manure, rocks, tree branches) present.

Sampling frequency for seedling monitoring varied with time from germination events. The emergence immediately following treatment application and a subsequent substantial rainfall event was the major germination event to be monitored through the year. Survival was defined as the young plants that lived through the first summer. Initial counts were done within 2-3 weeks of seeds germinating (after treatment application) and then after approximately 6, 24 and 52 weeks later. Each sample counted all the seedlings present and marked those newly emerged with coloured nails for monitoring of young plant survival.

After treatment application soil cores were taken across the site to determine the seed bank. Samples were only taken in those treatments where natural seed fall was assumed to differ, so the treatments that had addition of extra seeds were avoided. Within the plot, 2 soil cores (0.05 m diameter x 0.05 m deep) were randomly collected - one from each side of the 0.9 x 0.9 m area used for measurements. The cores for each plot were mixed, brought to the glasshouse, sifted, added to the surface of sand and placed into regularly watered pots. All seedlings that emerged were identified, counted, recorded and then combined into functional groups. The experiment continued until there were no more seedlings emerging, usually for a period of 2 weeks. An aim was to determine the readily germinable species that could compete with the emerging perennial grass seedlings.

Seed production across the site was estimated through each flowering season, which occurred over the summer. The total number of plants and seed heads in each of those plants were counted in 10 randomly selected 0.3 x 0.3 m quadrats across the site at the time of seed maturity. From these plants 10 seed heads were collected and seeds counted to estimate the amount of seed per seed head and total seed production.

The experiment was established in 2006 and *B. macra* seedlings were first observed during early autumn 2007 after summer rainfall during the months of January, February and March. The final count was made following the first summer after the initial germination event *i.e.* one year later in 2008. The first observations of seedlings were recorded on 14 March 2007 followed by young plant survival counts on 26 April 2007, 29 August 2007 and the final count on 18 February 2008.

Analyses

Differences between treatments in total standing biomass, green and litter biomass, functional group biomass, bare ground and plant cover at each measurement period were analysed by analysis of variance (ANOVA) using a split-split plot design. Differences in seedling numbers between treatments for each measurement period were analysed by ANOVA.

A General Linear Multiple Regression using a quadratic model (GLM) was used to identify associations of seedling recruitment and young plant survival with functional plant groups DM (t ha^{-1}), green DM (t ha^{-1}), litter DM (t ha^{-1}), bare ground (%) and plant cover (%). Sequential Bonferroni correction was used for alpha (α) level adjustment to account for multiple comparisons. Cross-correlations in regressions were investigated to avoid confounding of factors. Regression tree analysis using least squares was then used to investigate the relative importance of these factors on recruitment and young plant survival and identify the order in which factors were more important. No more than 10 splits were permitted and the terminal nodes had to contain 5 or more units. Minimum split and split proportions were set at 0.05.

Seedling data were square root transformed [√ (seedling m^{-2} + 0.5)] for all analyses. All statistical analyses were done using GenStat 9.1® (Payne *et al.* 2006) except the regression tree analyses that were done using Systat 12® (Systat Software Inc. 2007).

Results

Treatments were designed to create variation in the vegetation structure of the sward, viable seed levels and in suitable microsites for germination. The time of the identified recruitment event after treatment application was in March 2007 hence results are presented from late summer 2007 to summer 2008. The results for grassland description and species composition are presented only for the subset of treatments in grazing and pasture cropping and reducing competition as the treatments that modified seed levels did not affect the sward structure. The results on seedling recruitment and survival are presented across all subsets of treatments as seed level modification represented one of the most significant factors for emergence of seedlings.

Grassland description

In summer 2007 standing dry matter (DM) was higher in UG (0.88 ± 0.16 t ha^{-1}) than in PC (0.11 ± 0.16 t ha^{-1}) ($P<0.01$) (Fig. 2-a). GR (0.17 ± 0.16 t ha^{-1}) had lower standing DM than UG ($P<0.01$). The trend of UG with higher standing DM than GR or PC continued throughout the experiment except in spring 2007 when PC had the most biomass (4.21 ± 0.07 t ha^{-1}) ($P<0.001$) as a consequence of substantial crop growth by that time. No significant effect in total standing DM was observed from HA in comparison to NH. This was anticipated as the primary objective of HA was to kill any annual grasses germinating prior to *B. macra* germination events, but not greatly affect the standing perennial grass plants. The standing DM across all treatments was low (0.39 ± 0.18 t ha^{-1}) at the start of monitoring in summer 2007, but gradually increased through the experiment reaching 4.45 ± 0.33 t ha^{-1} by the end in summer 2008, except in PC which from spring 2007 to summer 2008 period decreased as a result of crop harvest in December.

Green DM was constantly lowest in PC ($P<0.05$) whereas UG had highest green DM until autumn ($P<0.001$) and from winter onwards GR had highest green DM ($P<0.05$) (Fig. 2-b). Green DM was very low (0.02 ± 0.02 t ha^{-1}) in summer 2007 increasing through the experiment until summer 2008 (1.87 ± 0.13 t ha^{-1}), except in spring 2007 when there was a slight decline across all treatments. Positive correlation of green DM with standing DM was observed across all treatments, except in spring 2007 (negative correlation) when the decline in green DM occurred (*data not presented*).

Litter DM increased across the treatments PC < GR < UG ($P<0.001$) throughout the experiment (Fig. 2-c). Across all treatments, litter was the highest in summer 2007 (1.48 ± 0.15 t ha^{-1}) then declined through winter (0.12 ± 0.01 t ha^{-1}) before increasing marginally in spring (0.34 ± 0.03 t ha^{-1}) and the following summer in 2008 (0.42 ± 0.05 t ha^{-1}). Winter 2007 was the season with the lowest litter DM in all treatments.

In summer 2007 most bare ground (66 ± 3%) was present in PC ($P<0.001$) which was significantly greater than GR (19 ± 3%) while UG (13 ± 3%) had the least ($P<0.001$) (Fig. 2-d). Bare ground declined during the experiment (from 32 ± 3% to 15 ± 3%) and there was no bare ground available in UG from winter 2007 onwards. In GR and PC, a small increase occurred in spring 2007 before declining until summer 2008. GR had no bare ground (total cover) in summer 2008. PC always had more than 40% bare ground present.

Plant cover was higher in UG (15 ± 3%) than GR (7 ± 3%) or PC (5 ± 3%) in summer 2007 ($P<0.05$); a trend that continued for the rest of the experiment (Fig. 2-e). This was the reverse situation to bare ground. A negative correlation was observed between plant cover and bare ground across all treatments (*data not presented*). HA had lower cover than NH ($P<0.05$) in summer 2007, but no significant difference was observed for the rest of the experiment. Plant cover steadily increased across all treatments through the experiment from an average of 9 ± 3% (summer 2007) to 64 ± 6% (summer 2008).

[Fig. 2]

Species composition

A total of 30 species was recorded at the site during the course of the experiment. The functional plant groups formed for analyses were *B. macra* (BM), annual grasses (AG), other species (OTH) and sown winter cereal crop. Species such as forbs, legumes, sedges, broadleaves which ranked low in BOTANAL were combined into a single functional group (OTH) otherwise there would have been very low and, or missing values limiting the analyses possible. *B. macra* dominated the species mix throughout the experiment except during the crop growth period in PC (Fig. 3). There was a gradual increase in *B. macra* DM through the experiment (0.37 ± 0.19 t ha^{-1} to 3.10 ± 0.53 t ha^{-1}) even though the proportion of total biomass in this group decreased until winter (from 95 to 37%) and increased afterwards (to 66%). *B. macra* had little or no growth through winter (GR had a slight decline). In summer 2007 UG (0.84 ± 0.16 t ha^{-1}) had significantly higher *B. macra* DM than GR (0.16 ± 0.16 t ha^{-1}) or PC (0.11 ± 0.5 t ha^{-1}) ($P<0.01$), and this trend continued through the experiment. AG were observed mostly in winter and spring 2007 and these exotic annuals were present more in GR and PC than UG ($P<0.05$). In winter 2007 there was around 30% AG (0.33 ± 0.11 t ha^{-1}) present, the highest for the experiment. Cereal crop (*T. aestivum*) was present only in PC treatment during winter and spring 2007.

The DM of a few C3 perennial grass species recorded during the course of the experiment was very low and not consistent enough to be included in the analyses.

[Fig. 3]

Recruitment patterns

Flowering and seed set

B. macra inflorescences were observed as early as November (late spring) and were present until March (early autumn), but most flowering occurred during the summer months with florets and seeds maturing unevenly over a period of several weeks. Maturation usually started at the uppermost tip of the seed head (inflorescence), progressing towards the base over a relatively short period (few days to a week). Small proportions of florets were harvested at a late maturity stage (late summer) when the flowering stems were reddish-purple in colour and the florets at the tip of the seed heads were just beginning to detach, that is when most florets would have reached maturity. These collected florets were used to calculate seed production (Table 1) and undertake germination tests. Seed weight averaged 0.98 ± 0.08 mg and the germination of florets immediately (1-2 weeks) after collection was around 35% at the optimal temperature of 30°C, suggesting that majority of seeds might be dormant.

Seed availability

No *B. macra* seeds were recorded in the soil seed bank during the experiment (Table 1). Nearly a third of the total germinating seed bank consisted of annual grasses (AG), more than half was clovers and the rest broadleaves. *B. macra* seed set before recruitment was reasonable (25.9 kg ha^{-1}; Table 1) but the low germination rate of fresh seeds (37% - Table 1) meant actual seed available was only 9.6 kg ha^{-1} (Table 2) for the identified recruitment event in the field. The result was a poor rate of recruitment (0.5% - 5 seedlings m^{-2}) across the experiment. Assuming that the seed addition treatment (22.8 kg ha^{-1} viable seeds) only added to what happened naturally, the gain was 1.9%, (Table 2). The best treatment without seed addition produced 38 seedlings m^{-2} whereas seed density reached 80 seedlings m^{-2} when seed was added (Table 2). Soil scarification through pasture cropping in the best treatment produced 20 seedlings m^{-2} from natural seed set whereas it substantially increased to 279 seedlings m^{-2} when seed was added (Table 2). This suggests recruitment is possible from natural seed set and that availability of microsites may pose a major constraint to recruitment.

[Table 1]

[Table 2]

General recruitment, overall young plant survival and treatment effects

B. macra seedlings emerged in early autumn 2007 (Fig. 4-i) after rainfall events in late summer to early autumn (Feb: 31 mm; Mar: 75 mm; Fig. 1). The highest seedling number (279 seedlings m^{-2}) was recorded in the plot where the treatment combination was PC x SA x HA. The greater the level of disturbance from UG to GR to PC, the higher the emergence of *B. macra* seedling numbers ($P<0.05$) (Fig. 4-i). PC had substantially higher seedling numbers than UG or GR ($P<0.001$). Emergence of seedlings was significantly increased by SA ($P<0.001$) with few seedlings recruiting in NS. Slightly more seedlings emerged in HA (17 m^{-2}) than in NH (12 m^{-2}), but this difference was not statistically significant ($P = 0.4$). SA did not have a significant effect within UG, but increased seedling numbers significantly within GR and PC ($P<0.001$) (Fig 4-v). SA x PC had the greatest seedling numbers ($P<0.001$). A third of the plots (16 out of 48) failed to record any seedling. Only 2 plots had more than 100 seedlings m^{-2} and the rest recorded densities between 1 and 100 seedlings m^{-2}. Seedlings of species other than *B. macra* were present across all treatments.

The most prominent were broadleaves (~44 seedlings m^{-2}) and AG (~20 m^{-2}). There were few legume seedlings (~1 m^{-2}) and *Trifolium subterraneum* L. accounted for most of these. Other than *B. macra*, there were no perennial grass seedlings observed. In relation to treatments, less AG seedlings were observed in GR than UG or PC and in HA than NH ($P<0.05$). There were fewer broadleaf seedlings in PC than GR or UG ($P<0.05$).

Emerged seedlings gradually declined in numbers and survival rates for young *B. macra* plants were very low during the 2007 drought year (Fig. 4-ii,iii,iv). On average, 24 seedlings m^{-2} emerged at the site which decreased to 7 seedlings m^{-2} by week 24 after emergence. Young plant mortality through the dry summer conditions was high with only on average 1 young plant m^{-2} still surviving at the end of the first summer, 52 weeks after emergence. The PC x SA x HA treatment combination that had the highest initial recruitment, also had the highest number of young plants (11 m^{-2}) surviving. The differences in seedling numbers across treatments remained the same at 6 weeks and 24 weeks, but by 52 weeks after emergence seedlings only survived in PC x SA treatments (Fig. 4). Seedling densities present at 52 weeks were not significantly different from zero, meaning that treatment effects could not be discerned. All the treatments declined in seedling numbers exponentially at the same rate through the year.

[Fig. 4]

Biophysical factors affecting recruitment

The imposed treatments altered the level of plant cover, bare ground, litter, biomass, green and functional group biomass. Treatment effects were significant hence the seedling numbers reflected changes in available microsites or resource space and competitive environment created in each treatment. These relationships are examined in this and the next sections. Data are only shown over the range in values of each data subset (per treatment) where a significant relationship was found. Within each significant relationship for the factor of interest zero values were deleted from the figures to improve clarity. Regression trees were used to determine the more important factors across all treatments. In these analyses of components, results are presented to investigate physical microsite descriptors (*i.e.* bare ground, litter, plant cover) for their influence on recruitment and competition (*i.e.* functional groups biomass). Within that framework each significant factor is described to identify the limits or to quantify the factors where greater seedling numbers were obtained.

Cross-correlations in regressions were investigated (*data not presented*) to avoid confounding of factors. Bare ground (%) was generally related to plant cover (%) but a high correlation was not found for the data when seedlings initially emerged in late summer 2007 (*data not presented*) although more plant cover (%) was associated with less bare ground (%). Therefore, both bare ground (%) and plant cover (%) were considered in these analyses. A significant ($P<0.001$; $R^2 = 0.42$) positive relationship was found between litter cover (%) and litter DM (t ha^{-1}). Because litter cover (%) compared to litter DM featured in less significant relationships in the initial analyses, litter DM was considered the more important factor and was used in these analyses. Litter DM was considered a more accurate measure due to corrections from calibration cuts whereas litter cover (%) data were based on visual estimates only. The combined area of litter cover (%) and bare ground (%) represents the space available for seedlings to emerge. As there was some overlap between litter cover (%) and plant cover (%) by definition, another variable (residual plant cover %) was generated by subtracting the sum of litter cover (%) and bare ground (%) from 100% and tested in preliminary analyses. A highly significant ($P<0.001$; $R^2 = 0.87$) positive correlation was observed between plant cover (%) and residual plant cover (%) hence only the direct estimate of plant cover (%) was used in the analyses. This also suggests that the overlap between plant and litter cover (both as %) was small and not of great concern in biasing analyses.

Initial seedling numbers were maximised (~20 m^{-2}) when litter DM was ~1.4 t ha^{-1} (Fig. 5-4 - PC that included NS x NH) or had lower maxima (~10 seedlings m^{-2}) at ~2.2 t ha^{-1} in GR that had NS x NH (Fig. 5-1). Collectively this suggests that litter DM needs to be ~1.4 to 2.2 t ha^{-1} to maximise initial seedling numbers by providing physical sites for recruitment.

Presence of bare ground in the 35-55% range maximised seedling numbers (~100 m^{-2}) within GR (Fig. 5-3 - that included SA x NH). Seedling numbers were at a lower maximum (~30 m^{-2}) when the bare ground range was narrowed to between 45-55% (Fig. 5-2 - GR that had NS x HA). This indicates there was no limitation on microsites within the grazed treatments when more than 35% bare ground was available. High numbers of seedlings were observed in the bare patches created through PC that had SA (Fig. 4-v), but the relationships between seedling numbers and bare ground within PC were not significant.

Use of regression trees across all treatments showed bare ground to be the most important factor determining overall seedling numbers (Fig. 6-a). Where bare ground was <35% average seedling numbers were 10 m^{-2} ($n = 281$) and >35% bare ground had on average 52 m^{-2} ($n = 151$) seedlings emerging.

Biophysical factors affecting young plant survival

24 weeks after emergence

Plant cover of ~35% resulted in the greatest number of young plants (~40 m^{-2}) surviving until 24 weeks after emergence (Fig. 5-8 - PC that included SA x HA) or whereas plant cover of <30% produced a lower maximum (~25 m^{-2}) in GR x NS x HA (Fig. 5-5). In these analyses plant cover was more significant than bare ground except in GR x SA x HA (Fig. 5-7) where 10-15% bare ground had the greatest number of young plants (~50 m^{-2}) surviving. However, a significant (P<0.001; R^2 = 0.53) negative correlation was observed between bare ground and plant cover across all treatments (*data not presented*) at this stage in late winter. Regression tree analysis across all treatments though showed bare ground to be the most important factor (as was during initial emergence) in determining overall numbers of young plants surviving 24 weeks after initial emergence (Fig. 6-b). More than 10% bare ground had on average 14 m^{-2} (n = 165) young plants surviving whereas <10% had only 2 m^{-2} (n = 267) young plants surviving.

The greatest number of young plants (~20 m^{-2}) were surviving when 0.8 t ha^{-1} annual grasses were present within GR that had SA x NH (Fig. 5-6).

52 weeks after emergence

Maximum survival number of young plants (~15 m^{-2}) at 52 weeks after emergence was recorded in the PC x SA x HA treatment when *B. macra* DM was in the 2-3 t ha^{-1} range in late summer 2008 (Fig. 5-9). However, regression trees using the combined data across treatments showed the biomass of the 'others' functional group (forbs, legumes plus sedges *i.e.* non-grass competitors) to be the most important factor in determining the overall number of young plants surviving 52 weeks after emergence (Fig. 6-c). The cut off value was 0.15 t ha^{-1}, below which 5 m^{-2} (n = 50) young plants were surviving and above which 1 m^{-2} (n = 382) young plants were surviving on average.

[Table 3]

[Fig. 5]

[Fig. 6]

Discussion

This study investigated the recruitment and survival of *B. macra* seedlings, a native perennial grass important for grazing livestock in south-eastern Australia, to better understand the mechanisms of a successful recruitment event. The approach taken was to investigate management options that encouraged seed set (by excluding grazing), prepared more suitable sites for seedling recruitment (by involving grazing or pasture cropping) and identified better post seed maturation and emergence tactics that aided young plant survival in the short to medium-term. Only one year of data was possible for the experiment as the site owner unexpectedly decided to use the land for other purposes.

A single recruitment event of *B. macra* seedlings was observed in early autumn after significant rainfall events in the second half of February and early March 2007 (~65 mm over 10 days; Fig. 1) which were the first major rainfall events after seed maturation. Hagon (1976) used data on the effect of temperature on germination and predicted *B. macra* would germinate best in spring whereas Moore (1958) suggested the species would establish best in summer. However, no seedlings were observed in spring or summer in this experiment, probably as a result of the lack of available moisture in spring and early summer or low average minimum temperatures (Fig. 1). Harradine and Whalley (1980) and Lodge (1981) reported that very few seedlings of native perennial grasses germinated in spring. This suggests that the better time for recruitment of perennial grasses would be in late summer to early autumn following seed set, a view supported by previous observations (Lodge 1981; Dowling *et al.* 1996). A suitable rainfall event needs to follow after seed set because it is well documented that grass seeds germinate and emerge only in the presence of adequate soil moisture (Wilson and Briske 1979; Maze *et al.* 1993; Hamilton *et al.* 1999; Zimmermann *et al.* 2008).

In this study soil cores kept in a glasshouse at optimal conditions for germination, recorded no perennial grass seedlings emergence over a 2-week period, although many other plant types did germinate and emerge (Table 1). Several other studies (Winkworth 1971; Mott and Andrew 1985; Silcock *et al.* 1990; Bertiller and Coronato 1994; O'Connor 1997; Lodge 2004; King *et al.* 2006) have shown that seeds of perennial grasses were usually scarce in the soil seed bank. A low germination percentage (37%; Table 1) for freshly collected seeds suggests a higher proportion of dormant seeds. Only 0.5% of viable seeds from seed set at the site recruited into seedlings (Table 2). No recruitment events occurred at other times of the year. The combined influence of low perennial grass seed reserves, low percentage of recruitment, possible dormancy mechanisms and a single window of opportunity for recruitment to occur indicate current seed set needs to be maximised to improve the chances of recruitment.

The results show that legumes were minor vegetative components of these pastures which perhaps suggest that the very dry seasons and/or low fertility may have been constraining the growth of legumes. However, the presence of legumes in these pastures and their potential effects in supporting livestock production and in nourishing the companion grasses by supplying a much needed nitrogen input if fertility constraints were alleviated could not be ignored. Given reasonable seasons and sufficient soil phosphorus and sulphur, legumes can be major contributors to pasture production and quality into the future.

The seed addition treatments had a large effect on the numbers of seedlings to emerge compared to the control treatment (Fig. 4-i). There was very little recruitment observed where there was no seed added. The positive effect of seed addition on emergence is in partial agreement with other experimental studies in perennial grasslands where seed has often been shown to be a major constraint to emergence (Fowler 1986a; O'Connor 1996; Hamilton *et al.* 1999; Wilsey and Polley 2003; Zimmermann *et al.* 2008).

The improvement in seedling numbers by disturbing the site was an expected result and is in general agreement with other studies (Kim *et al.* 1990; Hofmann and Isselstein 2004; Liu *et al.* 2008) which have shown that disturbance of the soil surface enhanced emergence and recruitment of perennial grasses by creating more potential microsites for germination. Farmers are less likely to apply seeds as was done in the experiment due to cost and limited availability of seed, hence maximising natural seed yield by encouraging flowering and seed set and creating suitable microsites (*e.g.* soil scarification) after seed set may be their only feasible option to increase the chance of recruitment.

Analysis of biophysical factors showed that the presence of some bare ground was one of the more important factors in improving *B. macra* recruitment (Fig. 5). Bare ground was expected to encourage seedling emergence and bare ground in the range of 35-55% maximised seedling numbers in this study. In ungrazed treatments, seedling emergence was minimal compared to grazed or pasture cropped treatments (Fig. 4-i), most probably because of competition and lack of open spaces through the dry year. Harper (1977) in an extensive review of literature found little evidence that germination was enhanced by vegetation cover. Lodge (1981) also found that seedling emergence and survival were highest for all perennial species that germinated in the open spaces between the bases of plant tussocks. This does not imply that there can be no recruitment near established plants, but the presence of established competitors may severely suppress seedling emergence as demonstrated in many studies on perennial grasses (Moloney 1990; Aguilera and Lauenroth 1993; Milton and Dean 2000; Zimmermann *et al.* 2008). Results showed that leaving *B. macra* pastures ungrazed produced a poorer recruitment than grazed, and if there is no pasture cropping then grazing combined with some physical disturbance may be the only realistic option to improve the chances of seedling emergence through a reduction in competition and the creation of potential microsites for germination.

It was anticipated that the presence of litter would maintain higher soil moisture levels and reduce the rate of drying at the soil surface (Evans and Young 1970; McWilliam and Dowling 1970; Mott *et al.* 1976) that would enhance germination (Fowler 1986b). Presence of litter (1.4-2.2 t ha^{-1}) was found to maximise *B. macra* seedling numbers in this study (Fig. 5-1,4). Lodge (2004) found that the presence of litter had a positive impact on the emergence of *P. aquatica* seedling, although the effect varied with time of the year and soil type. This suggests that strategic rest from grazing may be required if litter levels are low. In this experiment ungrazed treatments always had more litter than all grazed treatments (Fig. 2-c). Some suspect that with higher litter levels, populations of pathogenic pest such as slugs increase and kill off any emerging seedlings whereas other simply conclude that too much litter act as a physical barrier (Facelli and Pickett 1991) which prevents moisture reaching to the soil surface.

While successful initial recruitment was observed, survival of emerged *B. macra* seedlings was low through the year and most seedlings died during the first summer following emergence (Fig. 4-iv). Less than 30-35% plant cover was associated with higher numbers of young plants surviving during late winter at 24 weeks after emergence. Pasture cropped and grazed treatments helped deliver that better environment than ungrazed treatments where plant cover was usually much higher. This suggests intraspecific competition from neighbouring mature plants of the same species may have negatively affected seedlings because the phenology of mature plants of the same species would be synchronized with that of the developing seedlings (Harper 1977). Lodge (1981) identified intraspecific competition as a factor causing high mortality of native perennial grass seedlings.

Presence of annual grasses (~0.8 t ha^{-1}) did not have significant negative effects on surviving young plants at 24 weeks after emergence in late winter. The young *B. macra* seedlings that emerged earlier in the year would have had established sufficiently by then and were able to compete better against winter germinating annuals. This strengthens the view that late summer to early autumn is the ideal time for perennial grass recruitment because it increases the chance for survival as the presence of annual grasses is low and plants grow sufficiently to counter the more intense competition from annuals during cooler winter months.

While treatment effects on survival through to 52 weeks were not discernible, the number of young plants that survived that first year seems to have benefitted from the presence of competitive biomass of both perennial grasses and other functional groups that mainly included broadleaves and thistles. Again the pasture cropped treatment provided the better environment for seedling survival when the standing DM was ranged between 2-3 t ha^{-1} (as a result of grain harvest in early summer) compared to levels >5 t ha^{-1} in other treatments during late summer 2008 after 52 weeks from initial emergence. However, the high mortality may also be attributed mostly to prevailing drought conditions. Although there was follow up rainfall after seedling emergence, there was low precipitation in late winter to spring and hardly any rain fell in January (the hottest month of the year). The only significant rainfall occurred in December (148 mm) which was 3 times the average monthly rainfall (Fig. 1). From a study of seedling emergence and survival of *Aristida ramosa* in northern NSW, Harradine and Whalley (1980) found that seedling establishment in native pastures was controlled primarily by moisture availability. Seedling survival at 52 weeks from emergence was low, 1 m^{-2} on average with 11 m^{-2} in the best treatment. This clearly demonstrated that recruitment and survival was possible even in dry years. In wet years it can be argued that the improvement could proceed faster and future research may show that is probably the case.

In summary, seed availability and the presence of microsites are crucial factors to encouraging effective recruitment of *B. macra*. The results indicate there is a narrow window between maturation of perennial grass seeds and the occurrence of a suitable rainfall event in late summer for recruitment, before subsequent rainfall events and milder temperatures promote germination of annual grasses which cause substantial competition. The practical implication is that summer rest from grazing should maximise flowering and seed set as perennial grass seed bank is too low to produce cohorts of sufficient density to reliably restore the population dynamics of *B. macra* grasslands. Maximising the availability of seed will certainly improve the number of seedlings that emerge. However, some form of soil disturbance is required to create suitable microsites for seedlings to establish on this soil type. The pasture cropping treatment created this opportunity in the field experiment through mechanical disturbance. Light grazing after seed set could be a practical and low-cost alternative to encourage initial recruitment of seedlings. Though survival was low in the experiment some progress was achieved and future research on managing competition during early stages of seedling establishment may provide answers to improving the survival rates of *B. macra* seedlings.

Acknowledgements

The authors would like to thank Helen Nicol for statistical advice.

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Fig. 1 The monthly rainfall, and maximum (Tmax) and minimum (Tmin) temperature at *Bothriochloa macra* site plotted with the 60-year average, 1946-2005 (BOM 2008).

Fig. 2 (a) Total standing herbage DM t ha^{-1}, (b) green herbage DM t ha^{-1}, (c) litter DM t ha^{-1}, (d) bare ground percentage, and (e) plant cover percentage over time across UG (ungrazed), GR (grazed), PC (pasture cropped), NH (no herbicide), and HA (herbicide application) treatments in different seasons (every 3 months) for 2007-8 experiment; means from Analysis of Variance (ANOVA) used; standard error bars presented.

Fig. 3 The biomass (DM t ha^{-1}) of functional groups (*Bothriochloa macra*, annual grasses = AG, others and crop) over time across UG (ungrazed), GR (grazed), PC (pasture cropped), NH (no herbicide), and HA (herbicide application) treatments in different seasons (every 3 months) for 2007-8 experiment; means from Analysis of Variance (ANOVA) used.

Table 1 Average production of seeds at *Bothriochloa macra* site during the flowering periods of the 2007-8 experiment and seedlings (m^{-2}) germinated in the glass house from soil cores (50 mm deep) collected the start of the experiment in January 2007 when seeds were maturing; temperature range used was 30 / 15°C; total numbers (± standard error) in two weeks after sampling.

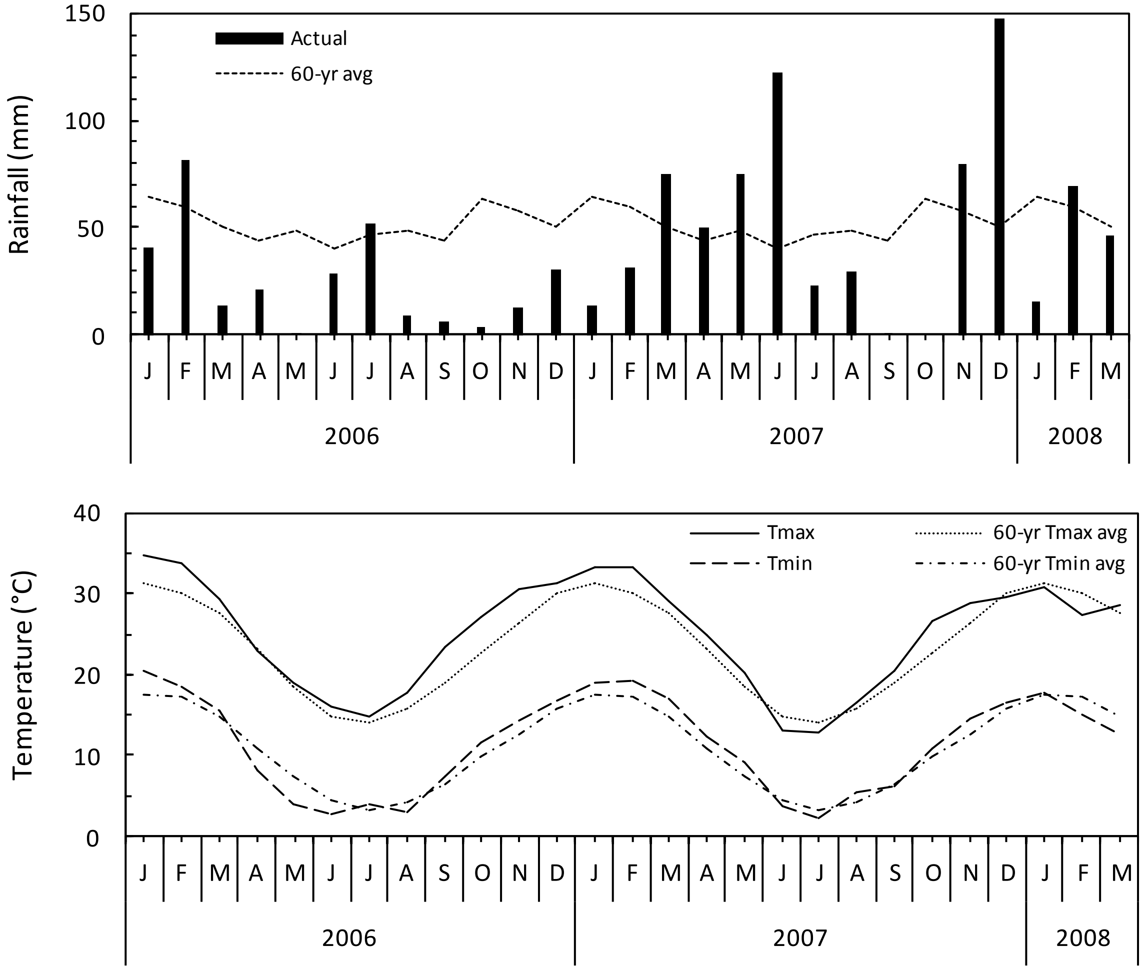
Table 2 Proportion of recruitment from natural seed set and extra seed addition across the experiment and the best recruitment with or without seed addition in any treatment and in soil scarification treatments through pasture cropping.

Fig. 4 The average number of *Bothriochloa macra* seedlings m^{-2} (logarithmic scale, n + 1) at (i) emergence, (ii) 6 weeks after emergence, (iii) 24 weeks after emergence and (iv) 52 weeks after emergence across broad treatment groups of UG (ungrazed), GR (grazed), PC (pasture cropped), NS (no seed), SA (seed addition), NH (no herbicide), HA (herbicide application) and (v) across all treatment combinations for all measurement periods; means from Analysis of Variance (ANOVA) used; for graphs (i)-(iv) within each subset of treatments at each measurement period, and for graph (v), within each measurement period, columns with the same letter are not significantly different, $P<0.05$.

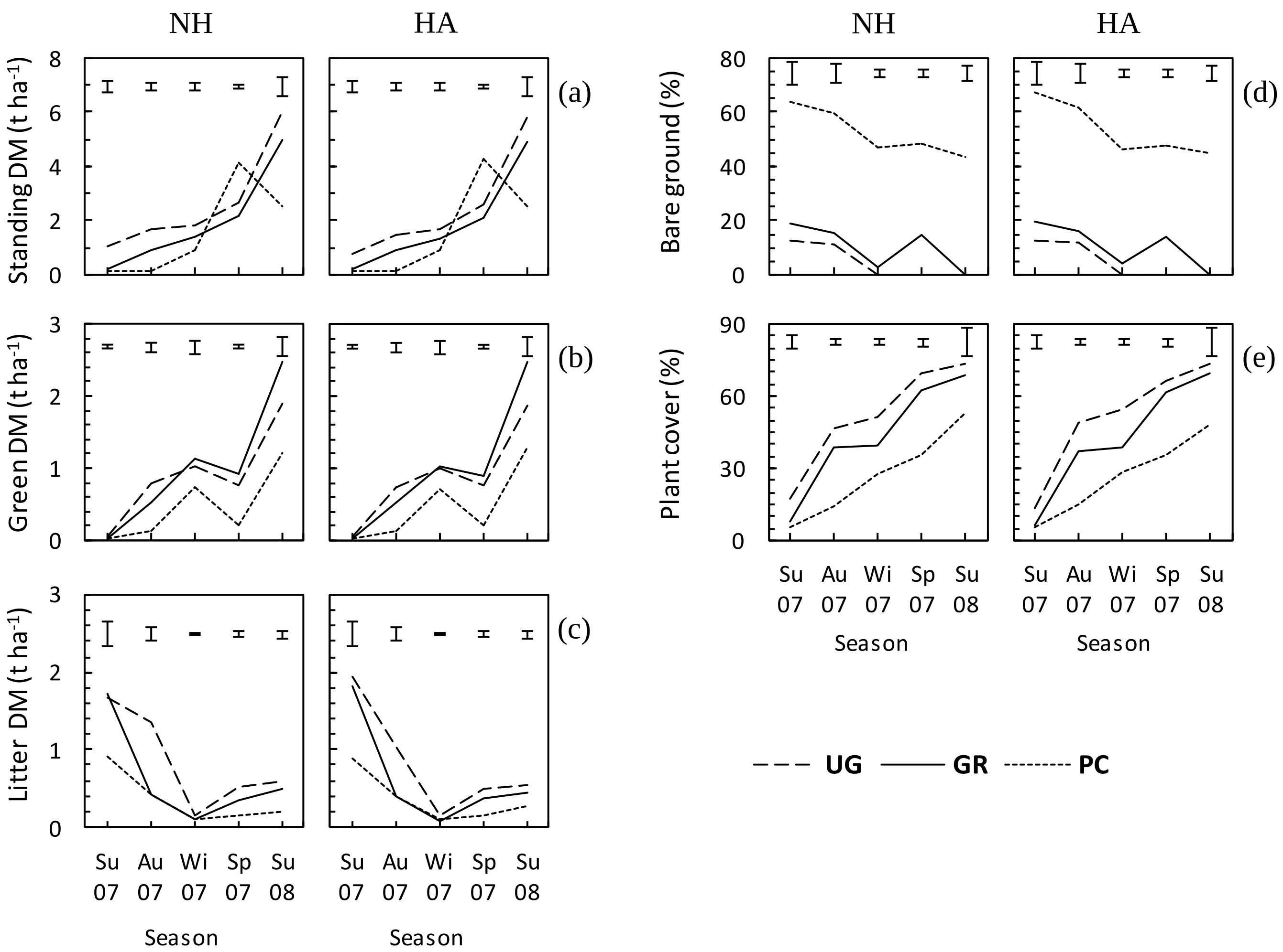
Table 3 General Linear Multiple Regression (quadratic model) equations predicting *Bothriochloa macra* seedling numbers across GR (grazed), PC (pasture cropped), NS (no seed), SA (seed addition), NH (no herbicide), and HA (herbicide application) treatments with significant relationships; where $SN = \sqrt{(\text{seedling number m}^{-2} + 0.5)}$; L = litter DM t ha^{-1}; B = bare ground %; AG = annual grass DM t ha^{-1}; C = plant cover %; BM = *Bothriochloa macra* DM t ha^{-1}; OTH = other functional group DM t ha^{-1}; the most significant factor was regressed against the number of seedlings (transformed) in Fig 5.

Fig. 5 *Bothriochloa macra* seedlings m^{-2} ($\sqrt{(n + 0.5)}$ transformed) compared to significant factors of General Linear Multiple Regression in Table 3; the solid line represents the most significant factor in the regression model; weak quadratic relationship not shown in graph 7; equation numbers from Table 3 correspond to individual graphs.

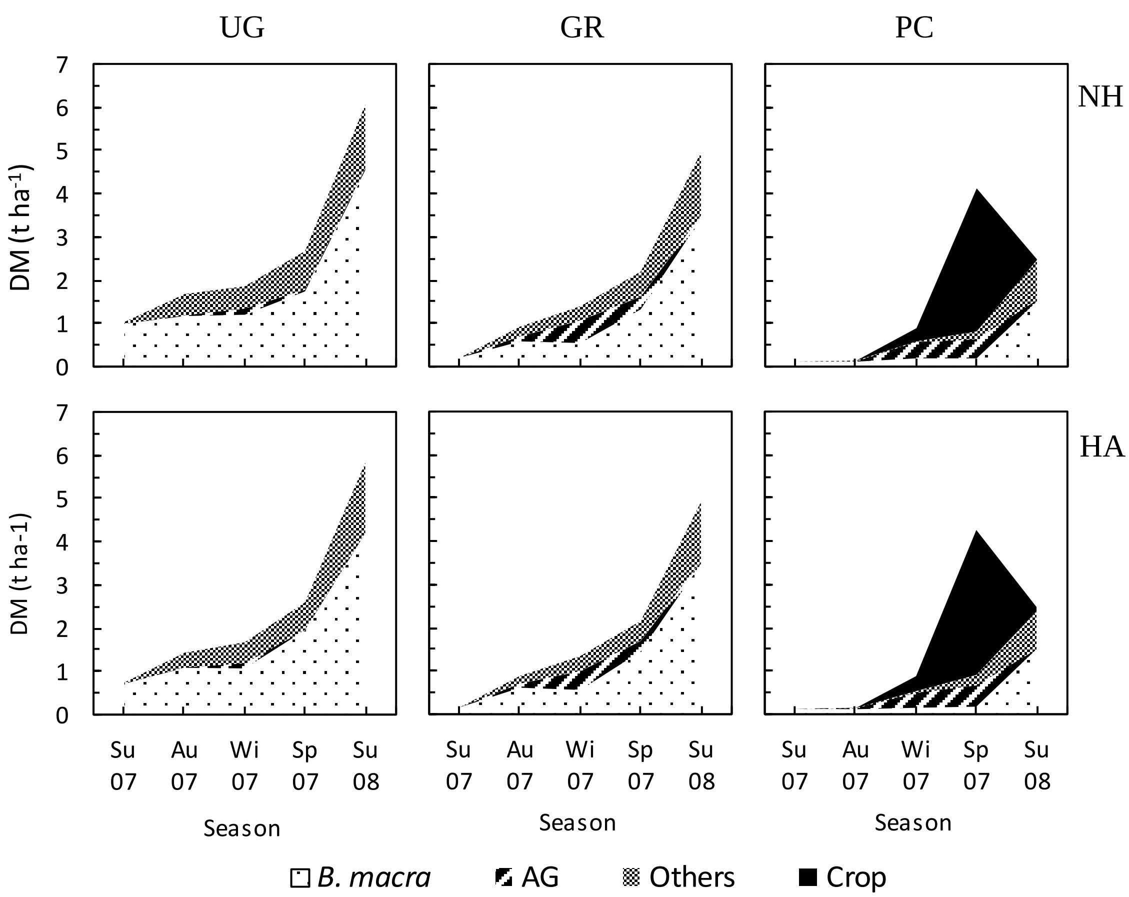
Fig. 6 *Bothriochloa macra* seedlings m^{-2} (a) at emergence in March 2007 as predicted by bare ground %, (b) at 24 weeks after emergence in August 2007 as predicted by bare ground %, and (c) at 52 weeks after emergence in March 2008 as predicted by others functional group DM t ha^{-1} across all treatments (standing DM t ha^{-1}, functional group DM t ha^{-1}, green DM t ha^{-1}, litter DM t ha^{-1}, bare ground %, plant cover % were initially included in the model). Proportional reduction in error (PRE) = 0.1.


Rainfall (mm)
Actual
60-yr avg
150
100
50
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M
2006
2007
2008
Temperature (°C)
Tmax
Tmin
60-yr Tmax avg
60-yr Tmin avg
40
30
20
10
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M
2006
2007
2008

1 Fig. 1


NH
HA
NH
HA
Standing DM (t ha^{-1})
Green DM (t ha^{-1})
Litter DM (t ha^{-1})
Bare ground (%)
Plant cover (%)
(a)
(b)
(c)
(d)
(e)
Su 07
Au 07
Wi 07
Sp 07
Su 08
Season
UG
GR
PC

1 Fig. 2



UG
GR
PC
NH
HA
DM (t ha-1)
DM (t ha-1)
0
1
2
3
4
5
6
7
Su 07
Au 07
Wi 07
Sp 07
Su 08
Season
B. macra
AG
Others
Crop

1 Fig. 3

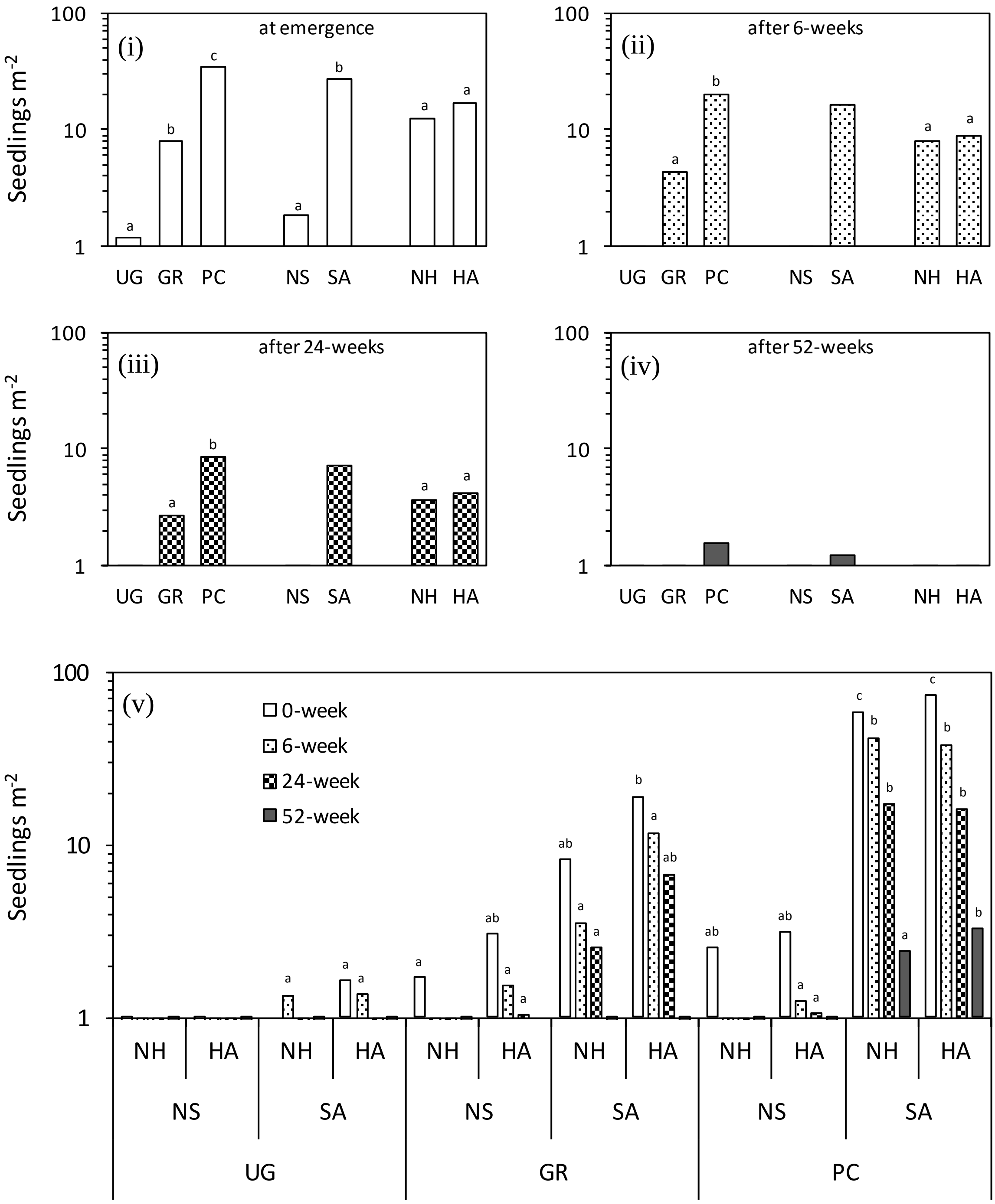
Table 1

	2007-8
Seeds m^{-2}	2640
Seed yield kg ha^{-1}	25.9
Germination (% at 30°C)	37
seedlings m^{-2}	
Bothriochloa macra	0
Annual grasses	2525 ± 710
Broadleaves	1676 ± 521
Legumes	4478 ± 2664

Table 2

Seed			Recruitment		Best recruitment (m^{-2})	
mode	amount		m^{-2}	%	any treatment	soil scarification
Natural	977 m^{-2}	9.6 kg ha^{-1}	5	0.5	38	20
Added	2156 m^{-2}	22.8 kg ha^{-1}	45	1.9	80	279

Note: The amounts of seed in column 2 were calculated from the data in Table 1 after accounting for germination percentages and the recruitment percentages in column 4 for the seed added treatments were calculated as the recruitment (natural - added) of the total added seed.

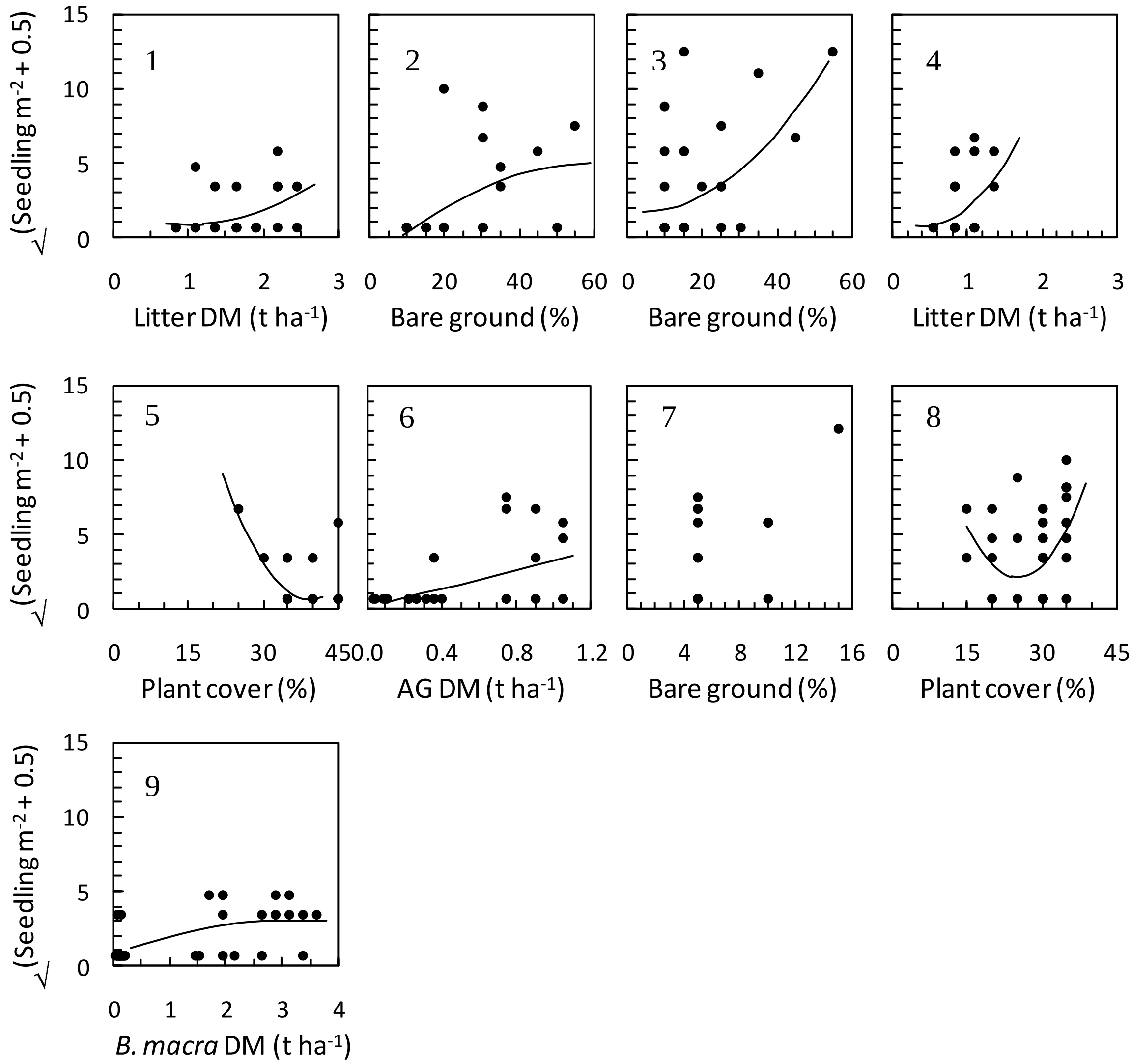


(i)
at emergence
(ii)
after 6-weeks
(iii)
after 24-weeks
(iv)
after 52-weeks
(v)
0-week
6-week
24-week
52-week
Seedlings m-2
100
10
1
UG GR PC NS SA NH HA
NH HA NH HA NH HA NH HA NH HA NH HA
NS SA NS SA NS SA
UG GR PC

1 Fig. 4

Table 3

Treatment			No	Equation	Adj R^2	P-value
At emergence (March 2007)						
GR	NS	NH	1	$SN = 1.9 - 1.91L + 0.94L^2$	0.16	0.02
		HA	2	$SN = - 1.66 + 0.22B - 0.0018B^2$	0.27	0.002
	SA	NH	3	$SN = 1.73 - 0.0.19B + 0.0038B^2$	0.29	0.001
PC	NS	NH	4	$SN = 1.32 - 2.79L + 3.5L^2$	0.16	0.02
Overall				$SN = - 6.3 + 0.14B - 0.00051B^2 + 6.51L - 1.53L^2$	0.17	< 0.001
At 24 weeks after emergence (August 2007)						
GR	NS	HA	5	$SN = 43.91 - 2.2C + 0.028C^2$	0.44	< 0.001
	SA	NH	6	$SN = 0.17 + 2.8AG + 0.29AG^2$	0.41	< 0.001
		HA	7	$SN = 1.64 + 0.039B + 0.037B^2$	0.39	< 0.001
PC	SA	HA	8	$SN = 23.02 - 1.66C + 0.033C^2$	0.16	0.021
Overall				$SN = 1.16 + 0.083B - 0.001B^2$	0.10	< 0.001
At 52 weeks after emergence (March 2008)						
PC	SA	HA	9	$SN = 0.84 + 1.35BM - 0.2BM^2$	0.33	< 0.001
Overall				$SN = 1.16 - 0.33OTH + 0.051OTH^2 + 0.025B - 0.00035B^2$	0.10	< 0.001


1
2
3
4
5
6
7
8
9
√(Seedling m-2 + 0.5)
Litter DM (t ha-1)
Bare ground (%)
Bare ground (%)
Litter DM (t ha-1)
Plant cover (%)
AG DM (t ha-1)
Bare ground (%)
Plant cover (%)
B. macra DM (t ha-1)

1 Fig. 5

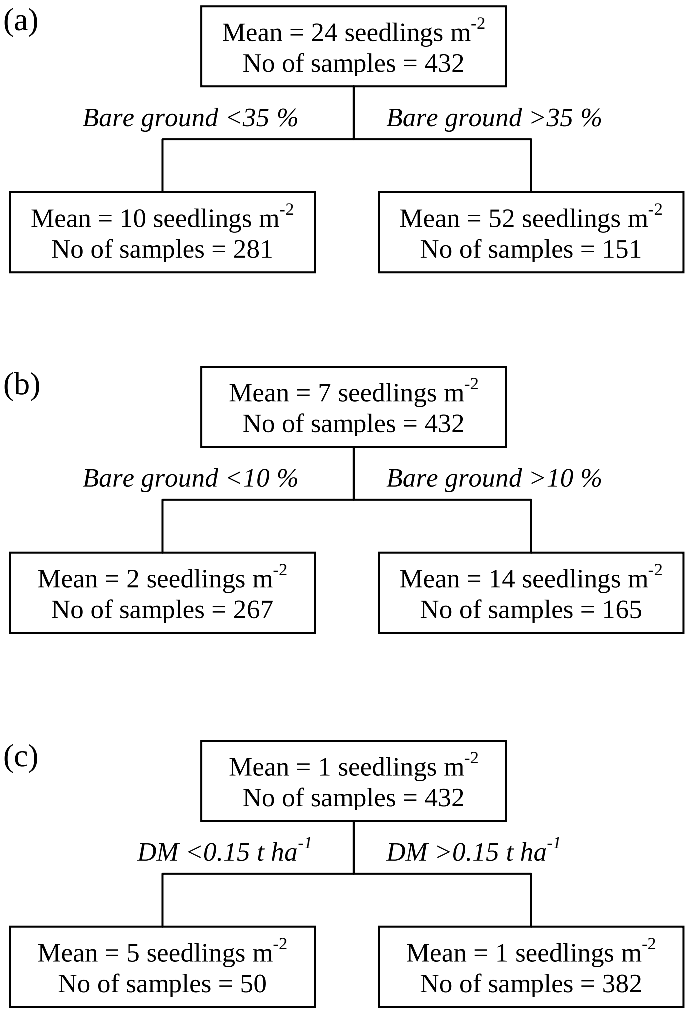

(a)
Mean = 24 seedlings m^{-2}
No of samples = 432
Bare ground <35 %
Bare ground >35 %
Mean = 10 seedlings m^{-2}
No of samples = 281
Mean = 52 seedlings m^{-2}
No of samples = 151
(b)
Mean = 7 seedlings m^{-2}
No of samples = 432
Bare ground <10 %
Bare ground >10 %
Mean = 2 seedlings m^{-2}
No of samples = 267
Mean = 14 seedlings m^{-2}
No of samples = 165
(c)
Mean = 1 seedlings m^{-2}
No of samples = 432
DM <0.15 t ha^{-1}
DM >0.15 t ha^{-1}
Mean = 5 seedlings m^{-2}
No of samples = 50
Mean = 1 seedlings m^{-2}
No of samples = 382

Fig. 6

Suitable climatic conditions for a seedling recruitment event within existing perennial grass swards in south-eastern Australia

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Short title: Climatic conditions for seedling recruitment

Abstract. Recruitment of new perennial grass plants within existing ecosystems is determined by seed availability, suitable microsites, resources and climatic conditions. This paper reports on the modelled soil moisture conditions associated with recruitment events in five field experiments at Orange (*Phalaris aquatica*), Trunkey Creek (*Austrodanthonia* spp.) and Wellington (*Bothriochloa macra*) in Central New South Wales, Australia, and the frequency of those conditions during the past 30 years. High seedling numbers were recorded when mature germinable seed was present and when a rainfall event (median 68 mm across the three sites) kept the surface soil moisture (0-50 mm) above the permanent wilting point for at least 15 days. Rainfall events typically occurred in the second half of February, sometimes extending to early March. No recruitment occurred if the surface soil was only moist for 7 days or less. The 30 years data from 1975-2004 showed that Orange had a median of 20 moist days each year at this time, whereas it was 16 days for Trunkey Creek and 10 days for Wellington. The probabilities of exceeding 7 or 15 days of moist surface soil were 98% and 78% at Orange, 91% and 49%

at Trunkey Creek, and 73% and 30% at Wellington. These results show some recruitment is possible in most years, provided seasonal conditions enable adequate seed set over summer. No further recruitment occurred on these sites from subsequent rain. Established seedlings survived the low rainfall period that then typically applied until winter. These analyses were extended for a number of sites in NSW, Victoria and Tasmania and showed some recruitment is possible and the principles developed have wider regional application.

Additional keywords:

soil moisture, modelling, recruitment event, drought, irrigation, perennial grasses

Introduction

The loss of perennial grasses from the grasslands of southern Australia and replacement by annual species - attributed to management practices (Moore 1970), has had severe implications for the productivity of livestock enterprises through weed invasion, erosion, salinity, acidity and nature conservation (Kemp *et al.* 2000; Michalk *et al.* 2003). Sustainability of these grasslands is of major concern to landholders, researchers and the community at large. Previous research has demonstrated that sustainable pastures are those that are based on perennial grasses (Kemp and Dowling 2000; Mason and Kay 2000). However, the current perennial content of many pasture systems is low, often accounting for ≤ 20% of pasture composition which is well below the level (60+%) that is desired for sustainability (Kemp and Dowling 2000).

This situation in permanent pastures across southern Australia is also evident in other parts of the world that have levels of perennial grass species, below the thresholds required to sustain ecosystem function. The long-term sustainability of these perennial pastures is dependent on the recruitment of plants of the same species or a similar species with a comparable function. Understanding how to encourage recruitment of new seedlings into existing swards is a substantial knowledge gap in grassland ecology. Limited research has shown that there is often low or nil survival of desirable grass seedlings in existing paddocks which may be largely due to the fact that perennials rely mostly on vegetative reproduction than on reproduction through seeds/seedlings. In a study of seed dormancy, germination, seedling emergence and survival of perennial pasture grasses in northern New South Wales, Lodge (2004) posed the challenge of identifying the causes that limit recruitment. This clearly highlighted the need for more research to devise appropriate and practical management options to encourage the emergence and survival of new seedlings into pastures. The study of Lenz and Facelli (2005) in South Australia on recruitment of both native perennial grass and exotics further identified the need for research to look into the reasons behind the low survival of perennial grass seedlings.

Recruitment from seed which is a natural event that occurs within ecosystems is determined by the availability of seed, suitable microsites, other resources and climatic conditions. Seed availability and soil disturbance, either occurring naturally by self-mulching processes in soil or through management interventions such as soil scarifying or grazing to create suitable microsites are important to maximise recruitment (Thapa 2010). The key climatic conditions required include suitable temperature and adequate soil moisture. Soil moisture available to the seed is one of the main factors that enables germination and establishment of perennial grass seedlings (Dowling *et al.* 1971; Fowler 1986; Maze *et al.* 1993; Lauenroth *et al.* 1994; O'Connor 1996; Zimmermann *et al.* 2008).

Recruitment occurred at all the field sites at Orange (149°07' E, 33°14' S), Trunkey Creek (149°19' E, 33°49' S) and Wellington (148°58' E, 32°30' S) in central New South Wales (NSW) of Australia (Thapa 2010). This study focused on native and introduced perennial grasses that are widespread and important for grazing in the region. Using the data obtained from these three field sites, combined with a small plot study using irrigation and seed addition through the year, this paper aims to first identify the soil moisture conditions associated with initial recruitment events and then to predict the frequency of attaining those required conditions using climatic data for the 30 years from 1975-2004. Historical climate data from a number of sites across NSW, Victoria and Tasmania were also analysed to determine the probability of obtaining suitable soil moisture conditions (as determined from the field sites) for a recruitment event to occur. Emphasis is placed on the initial seedling recruitment conditions as the drought years resulted in a low frequency of survival of young plants which proved to be insufficient to extend the climatic analysis to the conditions required to support plant survival through the subsequent summer.

Methods

Field experiment data sets used

Recruitment events were observed in each of the 5 experiments: 2 each at Orange and Trunkey Creek, and a single event at the one Wellington experiment despite the prevailing drought conditions (Thapa 2010). The seedling recruitment events observed for *Phalaris aquatica* L. (at Orange), *Austrodanthonia* spp. H. P. Linder (at Trunkey Creek) and *Bothriochloa macra* (Steud.) S.T.Blake (at Wellington) occurred in March (early autumn) after significant rainfall events in the February-March period. Rainstorms in late February at all the sites were the first major event after seed maturation and treatment application. Seedlings observed in March resulted from these February rainfall events. No other significant recruitment events occurred during the years of the research (Thapa 2010). These February rainfall events and the resulting estimates of moisture in the top 50 mm of the soil surface layer were carefully analysed to determine the soil moisture conditions when the 5 recruitment events occurred. A soil depth of 50 mm was chosen as root growth would not extend much beyond that within the first few days after germination.

Irrigation experiment

The irrigation experiment was done at the same field sites in Orange, Trunkey Creek and Wellington. The experiment started in January 2007 and continued until February 2008 except at Wellington where the experiment was cut short in October 2007 due to the unavailability of the site. Water was applied at approximately 6 week intervals at a rate equivalent to 50 mm rainfall (50 L m^{-2}) over 2 days at 25 mm on each day. Separate plots were used on each occasion. Initially no perennial grass seed was added at each site to test recruitment from the seed bank. However, due to a lack of seedling emergence and accumulated evidence that the perennial grass soil seed bank was presumed to be exhausted (Thapa 2010), the design was modified to add the equivalent of 50 kg seed ha^{-1} to the watered plots from November 2007 and continued until the end of the experiment in February 2008. Seed addition could not be done in Wellington as the site was unavailable by that time. Of the total of 21 watering events across the three field sites, 13 coincided with rainfall events.

Plots were 1 x 1 m with a 1 m buffer between individual plots and were replicated 3 times. A 1 x 1 m galvanised metal plate (0.15 m high) was used to retain water within the 1 x 1 m treatment area. Plots were covered with shade cloth for 2-3 days following irrigation to reduce evaporation. For each watering period new plots were established and watered. Sampling was done within a 0.9 x 0.9 m area in the centre of the 1 x1 m plot. The 0.9 x 0.9 m area was subdivided into nine 0.3 x 0.3 m contiguous quadrats in which pasture yield and composition was measured after each watering using BOTANAL procedures (Tothill *et al.* 1992). Two quadrats (one for high and one for low emergence) were selected from the 9 quadrats for seedling recording. Each of these 0.3 x 0.3 m quadrats was further divided into 36 sub-quadrats (each 0.05 x 0.05 m) and used for seedling recording. All seedlings within the 0.05 x 0.05 m sub-quadrats were counted *i.e.* a total of 72 sub-quadrats and identified approximately 2 weeks after each watering. Within the buffer area adjacent to the plot, 2 soil cores (0.05 m diameter x 0.05 m deep) were randomly collected - one from each side and grown for a 2-week period in the glass house for soil seed bank studies during each watering period.

Climate data

Climate data were measured using data loggers (Tain Electronics®) at the site for the duration of the experiment. Soil temperatures and moisture (using gypsum blocks) in the soil surface layer (0-50 mm) were recorded as well as relative humidity, air temperature, solar radiation, wind speed, and rainfall. At Wellington, the site for the *B. macra* experiment, rainfall data were obtained from the weather station located <0.5 km from the experimental site. The long-term climate data were obtained from the National Climate Centre of the Australian Government Bureau of Meteorology (NCC 2009).

Soil moisture model

The percentage volume of moisture in the top 50 mm soil surface for the experiment duration and for the 30 years from 1975-2004 were estimated using the Sustainable Grazing Systems (SGS) pasture model (Johnson *et al.* 2003), version 4.5.4 (Johnson 2008). The SGS model uses daily climate data (rainfall, temperature, relative humidity, wind speed, vapour pressure, evaporation, solar radiation), soil physical properties, soil nutrient based on the initial inorganic status (NO_3 and NH_4), pasture species and latitude to calculate soil moisture values for the period defined in the model. When data was not available for parameters, the model used the generic patterns based on the latitude, longitude and other relevant factors. The soil physical properties were used for the generic soil types. The analysis focused on the soil moisture status in the top 50 mm created by the rainfall events before the time of the identified recruitment events. At the start of each model run the soil moisture levels were considered to be zero as an extended dry period was chosen as the starting period.

Additional sites for climate analysis

Several sites in a north-south transect through south-eastern Australia were investigated to determine if the principles developed would apply in those areas. These sites were Armidale, Bathurst, Bombala, Canberra, Cooma, Goulbourn, Mudgee and Tamworth in New South Wales (NSW); Beechworth, Creightons Creek, Highlands, Mansfield, Tallangatta, Tambo Crossing and Warrenbayne in Victoria; and Avoca, Cressy, Dunalley, Hobart, Launceston and Swansea in Tasmania. All sites had moderate (~600-800 mm) annual average levels of rainfall. Rainfall events from late summer, through autumn occur in many of those districts, but it was not clear what the probability of sufficient rainfall was to enable germination to occur. The grasses studied are known to exist throughout the south-east and do naturally recruit. The assumption in this work was that the events and timing that resulted in recruitment of seedlings in field sites would apply across the region.

Results

February - March rainfall

Five recruitment events were observed across the three field sites and each occurred after rainfall events in February that in some instances extended into early March. Soil moisture in the top 50 mm was estimated during and after those rainfall events using the SGS model. After initial modelling of soil moisture status and reviewing the associated recruitment events, it was decided to identify the periods where the soil moisture content was between ~40% (*i.e.* close to field capacity in these soils and where free water is available for a seedling to emerge) and ~20% (*i.e.* close to the permanent wilting point where there would be little available moisture for a seedling). While actual values for field capacity and permanent wilting points may not be exactly at 40 and 20% across the sites, they were close enough to estimate how long the soil would have some available moisture for seedling growth. The values used varied between sites. For example, if the estimated soil moisture was above or near 20% and then not changing that suggested it was very close to the permanent wilting point and plants had limited capacity to extract much water. In these cases the soil was then considered to be effectively too dry for seedling growth.

In general, across the sites there were 2 close rainfall events in the February-March period (Fig. 1) which increased soil moisture in the top 50 mm closer to field capacity (~40%). These rainfall events preceded the identified recruitment events in March. The soil moisture estimates showed these two rainfall events were usually separated by a maximum of 2 dry days. Since sampling for recruitment observations was made after the second rainfall event and daily measurements of recruitment were not taken, it is not possible to determine if there was any seedling mortality during these apparently drier days between the 2 rainfall events in the February-March period. Of these two events, the first event could prime the seeds for initial emergence and then the second event could establish the seedlings. For analytical purposes, the total of these 2 events was considered to be the significant rainfall event that resulted in seedling emergence.

Recruitment events in the main field experiments

Recruitment event 1 (*Phalaris aquatica* 2006)

The first observation of *P. aquatica* seedlings was made on 7 March 2006 in Experiment 1. The rainfall event preceding this recruitment event started on 15 February and ended on 18 February with a total of 33 mm over 3 rain days (Fig. 1-a). As a result soil moisture was predicted to have reached 43% on 15 February which declined to 24% (23 February) before levelling off. This set up the conditions for some seedlings to emerge as the soil was moist for around 9 days. The soil was then dry for 2 days before a second rainfall event on

26 February for 2 days (24 mm) that was predicted to have raised the soil moisture content to 42% and kept the soil moist above wilting point for another 9 days until 6 March. Collectively this suggests the rainfall between 15 and 27 February (57 mm over 5 rain days) resulted in moist soil surface conditions for a total of 18 days over the whole 20 day period (15 February to 6 March) which generated the seedlings recorded on 7 March. The 2 dry days in the soil surface during the wet periods evidently did not affect recruitment.

Recruitment event 2 (*Phalaris aquatica* 2007)

P. aquatica seedlings were first observed on 21 March 2007 in Experiment 2; about 2 weeks later than Experiment 1. The preceding rainfall event occurred from 23 February to 1 March (54 mm over 6 rain days), the effect of which lasted for 8 days (23 February to 3 March) where the soil moisture gradually declined from 47 to 23%; this resulted in the emergence of seedlings and was further assisted by another rainfall event starting on 5 March (Fig. 1-b). There were 5 continuous rain days (45 mm) with soil moisture lasting for 8 days until 12 March (43 to 21%). In total, there were 99 mm rainfall over 11 days keeping the soil moist for 16 days over the total period of 18 days that resulted in the recruitment event in the third week of March. The model estimates suggested that the 2 dry days during this period in surface moisture content were not deleterious to recruitment.

Recruitment event 3 (*Austrodanthonia* spp. 2007)

Initial recruitment of *Austrodanthonia* seedlings was observed on 13 March 2007 in Experiment 1. The estimated soil moisture content reached 38% on 18 February and remained above ~20% for the next 6 days due to 35 mm rain falling over 4 subsequent rain days (Fig. 1-d). The next immediate rainfall event happened 2 days later, from 26 February to 28 February (33 mm over 3 rain days) resulting in moist soil conditions (≥20%) until 2 March. The soil moisture conditions that triggered the recruitment event existed between 18 February and 2 March with 68 mm rainfall across 7 days which had the soil moist for

11 out of the total period of 13 days. Again, the 2 dry days in the middle of this rainfall sequence did not seem to have limited recruitment in general.

Recruitment event 4 (*Austrodanthonia* spp. 2008)

Austrodanthonia spp. seedlings were recorded on 10 March 2008 in Experiment 2 which was one of the less successful recruitment event recorded. There were 2 rainfall events in February that would have enabled the emergence of seedlings (Fig. 1-e). The first occurred on 1-8 February (59 mm over 3 days) increasing soil moisture to a peak of 44% and was >~20% for 9 days. The second event occurred on 12 February which kept the soil moist for 4 days from 18 mm of rainfall received over 2 days. The combination of these two events (77 mm rainfall over 5 days which kept the soil moist for 13 days) created suitable moisture conditions for the recruitment event recorded in the second week of March. The estimated 2 dry days between the events again did not seem to affect recruitment. There was a third rainfall event on 28 February (17 mm over 2 days) before the sampling for the recruitment was made but this event was considered less important in the analysis as the earlier events essentially created the appropriate soil moisture conditions for seedling emergence.

Recruitment event 5 (*Bothriochloa macra* 2007)

The identified recruitment event for the *B. macra* experiment occurred on 14 March 2007. The rainfall event started on 26 February and ended on 1 March with 32 mm over 4 rain days (Fig. 1-f). As a result, the soil remained moist for 6 days until 4 March which possibly initiated the first instances of seedling emergence. The next event from 5 to 8 March (33 mm over 3 rain days) kept the soil surface moist for 8 days and further assisted the recruitment event. The combined rainfall event from 26 February to 8 March (65 mm over 7 rain days) maintained adequate soil moisture for 14 consecutive moist days, stimulating seedling recruitment.

[Fig. 1]

Recruitment events in the irrigation experiments

The six weekly irrigation treatments used across the three sites did not result in any significant seedling recruitment on most occasions even though viable grass seeds were detected in the soil cores. There were 21 watering events across the three field sites, of which 8 events recorded recruitment - 3 at Orange, 5 at Trunkey Creek and 0 at Wellington. Of the 8 recruitment events, 6 had both water and seed applied and coincided with the rainfall events. The plots of the irrigation experiments resembled closest the control plots +/- seed addition of the main field experiments across the sites as no treatments were applied other than water and seed.

At Trunkey Creek 13 seedlings m^{-2} were observed on 5 February 2007 when water was added without seed addition. This event coincided with a rainfall event in mid January 2007 which extended the period of soil moisture to 10 days (Fig. 1-d) and some current mature seed may have been available. The irrigation treatment in late February 2007 failed to generate any seedlings in early March when rainfall events that coincided with irrigation extended the period of soil moisture to 10 days. The control plots of the main experiments had only 2 seedlings m^{-2} across the site at the same time. It was found *Austrodanthonia* spp. emerged mostly where seed was available and site disturbed through soil scarifying (Thapa 2010). The irrigated plots did not have both at the time. A single recruitment event (2 seedlings m^{-2}) occurred in mid September 2007 as a result of irrigation applied without seed at the end of August 2007 and did not coincide with any rainfall. Low recruitment occurred in mid November 2007 and early January 2008 where seed was added and the events coincided with rainfall. Limited recruitment (9 seedlings m^{-2}) occurred in early March 2008 with seed addition from irrigation in late February when 17 mm of rain fell shortly afterwards. This was not significantly different to the main field experiments as seed added control plots of the main field experiments recorded 5 seedlings m^{-2} across the site (Thapa 2010). The result was similar at Wellington with *B. macra*. The general lack of recruitment in irrigated treatments reinforces the view that native grass recruitment depends predominately on seed availability, microsites and adequate soil moisture conditions at critical times.

At Orange all 3 observed recruitment events in irrigation experiments had seed added and coincided with rainfall which extended the period of soil moisture to 16-23 days. More seedlings (11 m^{-2}) were found in early March 2008 from the irrigation treatment in late February. The watering treatment that coincided with rainfall events in late February 2007 did not result in any recruitment whereas the control plots of the main experiments had 16 seedlings m^{-2} at the same time (Thapa 2010). The irrigation treatment only had half the herbage mass (t DM ha^{-1}) compared to the control plots of the main experiments at that time. More *P. aquatica* seedlings were found to be associated with higher standing herbage mass (Thapa 2010).

The irrigation treatments provided soil moisture conditions that did not often result in recruitment. While irrigation may have resulted in the priming of seeds, the lack of follow-up rainfall meant that recruitment did not eventuate. Estimates of surface soil moisture conditions in the irrigation treatments (50 mm over 2 days) showed in most instances that when watering did not coincide with any rainfall, the soil (0-50 mm) was moist for only about 5 days. When the watering did coincide with a rainfall event, the soil remained moist for an average of 24 days across the sites and resulted in some recruitment within the irrigation experiments.

Soil moisture conditions

The soil moisture level immediately before the recruitment events in the main field experiments and the irrigation experiments were used to determine the minimum and maximum conditions required to produce acceptable recruitment. The rainfall events in January and earlier months were not considered for analysis as they occurred before seed maturation and seed fall in most instances, and in the main experiments no recruitment was observed at this time. The treatment application in the main field experiments occurred mainly in the month of January and the next significant rainfall event was not until late February or early March. Since the significant recruitment events in the main field experiments occurred only in early March across all the sites emphasis was on identifying the soil moisture conditions needed to achieve a recruitment event from rainfall events during February and March.

Minimum soil moisture conditions

The irrigation experiments aimed to generate an adequate soil moisture level for a satisfactory recruitment event by applying irrigation equivalent to 50 mm rainfall over 2 days at 25 mm on each day. Recruitment was minimal and hence the results were used to set minimum moisture conditions needed for a recruitment event to occur. In most instances, recruitment was not observed and in few instances when recruitment events occurred, the irrigation applications mostly overlapped the rainfall events thereby extending the days for the soil surface layer (0-50 mm) to remain moist and when extra seeds were added. Across the sites there were 6 irrigation events that did not coincide with the rainfall events and had no seed added. As these 6 irrigations did not result in recruitment, the resulting soil moisture conditions in these events were considered inadequate for a recruitment event. On average, the model estimated that soil in the top 50 mm was moist for 7 days in these 6 irrigation events which was not enough for a recruitment event to occur. Therefore, this period of moist surface soils (*i.e.* 7 days) provided the minimum criteria below which recruitment was highly unlikely.

Ideal soil moisture conditions

Recruitment in the main field experiments across the three sites resulted from rainfall events in February, early March. Often there were 2 close rainfall events separated by 2 dry days before the recruitment occurred in the field. Those 2 dry days did not appear to have any adverse effects. The resulting soil moisture conditions from these rainfall events in the February-March period were considered adequate for a recruitment event to occur. On average across the sites the rainfall events were 68 mm falling over 7 days or more which kept the soil moist for at least 14 days. This provided the criteria for ideal number of days (*i.e.* 14 days) that the soil needed to be moist for a recruitment to occur.

Past climate data and frequency of recruitment

Field sites

Plant recruitment depends more on the number of contiguous days of soil moisture availability and never rainfall *per se*. Climate data recorded close to each of the field sites were analysed to determine the duration of a significant rainfall event that kept the soil in the top 50 mm moist (~40-20%) in the months of February and March over the last 30 years (1975-2004). The significant rainfall event was identified based on the experiment results and where applicable included a maximum of 2 dry days in the period as per the main field experiments. This information was then used to estimate the probability of achieving adequate soil moisture conditions in any given year for a recruitment event to occur.

Across the three sites a recruitment event in the main field experiments was achieved when the soil in the top 50 mm was moist for around 14 days that maintained adequate soil moisture conditions. Based on these experimental results 15 days of adequate moisture was set as the safe criterion that should result in a high possibility of recruitment occurring. The irrigation experiments helped identify 7 days as the minimum numbers of days the soil moisture was inadequate and where there was a low probability of recruitment occurring. Midway between the minimum and ideal conditions are arguably the events that will produce noticeable improvements in the recruitment of these desirable perennial pasture grasses.

Analyses for the past 30 years showed that Orange had an average of 20 moist days each year following a satisfactory rainfall event in the February-March period, whereas it was only 16 days for Trunkey Creek and 10 days for Wellington (Table 1). On average some recruitment is then possible. The shortest moist period was 2 days at both Trunkey Creek and Wellington, and 9 days at Orange. The longest moist period was 31 days at Orange and 24 days at Trunkey Creek and Wellington.

The average rainfall coinciding with 15 days of soil remaining moist was 80 mm for Wellington (Fig. 2-3) but only 48 mm for Orange (Fig. 2-1) and Trunkey Creek (Fig. 2-2) though in each case there was considerable variation. These values are less than those identified from the experiments of 57-99 mm. This reinforces the point that the number of days of moist soil is the more important criterion which determines the success or failure of a recruitment event. All sites showed a curvilinear relationship between days of moist soil from the associated rainfall event (Fig. 2-1,2,3). This suggests that larger rainfall events tended to be more intense and did not add greatly to soil moisture because it fell over shorter time periods. This effect was most noticeable at Trunkey Creek (Fig. 2-2).

Further analysis from modelling 30 years of data showed Orange should get some recruitment every year as the probability of exceeding 7 days of moist soil was 98% and 78% of years exceeded 15 days (Table 1). Trunkey Creek would only fail in 9% of years to achieve the minimum moisture conditions of 7 days and 49% of years should have a high chance of recruitment. The worst site was at Wellington where 73% of years would result in little recruitment, with only 30% of years experiencing at least 15 days of moist soil.

Over the last 30 years Orange experienced the first significant rainfall event after seed maturity, from 1st February usually in the second week of February whereas Trunkey Creek and Wellington typically experienced the preferred rainfall event in late February, but still averaging 15 days (Fig. 3-1,2,3). As Trunkey Creek and Wellington are hotter climates than Orange this meant that temperatures were similar at all sites during the moist soil periods. In some years the first significant rainfall of the year did not occur until later in March. Such events could still prove successful for recruitment as temperatures at this time are still close to 20˚C, though more competition from annual grasses would occur at this time of the year (Bowcher 2002). These analyses did not extend into April as often weed competition is more prevalent at that time.

Additional sites

The field experiments determined 7 and 15 days of continuous soil surface moisture as the minimum and ideal conditions for a recruitment to occur. Using the same principles, investigation of the climate data for the sites across NSW, Victoria and Tasmania showed some recruitment is possible, provided seasonal conditions enable adequate seed set over summer. Each of these sites has natural perennial pastures, which indicates that recruitment does occur there at times. In most instances across the sites and the years, there was a single significant rainfall event during February-March period that kept the surface soil moist for 7-15 continuous days. The probability of exceeding 7 days of moist surface soil (*i.e.* minimum soil moisture conditions for a recruitment to occur) was more than 55% across all sites *i.e.* at least one year in two; the range being from 92% at Armidale to 56% at Creighton's Creek and Avoca with other sites in between (Table 1). The probability of obtaining ideal soil moisture conditions (*i.e.* 15 days of moist surface soil) was lower. The highest was at Armidale (39%). Two sites, Creighton's Creek and Highlands had 0%

probability of exceeding 15 days of moist soil though they would still experience somewhere between 7-15 days. Remaining sites had some chances (3-35%) of obtaining 15 days of moist soil. Median days of moist soil each year at this time (February to March) across the sites were between 8 and 14. Average rainfall at median, 7 and 15 days of moist soil was between 22-67 mm, 17-41 mm and 37-94 mm (Fig. 2; Table 1). Majority of the sites investigated experienced the preferred rainfall event in late February early March (Fig. 3). Sites such as Cooma (Fig. 3-8), Creightons Creek (Fig. 3-13), Mansfield (Fig. 3-15) however had the rainfall event uniformly spread across February-March period.

[Table 1]

[Fig. 2]

[Fig. 3]

Discussion

Earlier research on sown temperate perennial grasses in Australia (Lodge 1979; Dowling *et al.* 1996; Waller *et al.* 1999; Virgona and Bowcher 2000; Lodge 2002; Lodge 2004) has suggested that recruitment is infrequent. Initial recruitment was achieved at each of the three sites despite prevailing drought conditions, though survival rates of young plants was low (Thapa 2010). If rainfall was higher then greater number of initial recruiting seedlings may have been recorded. The graphs of soil moisture in the top 50 mm (Fig.1) show that in all years, soil moisture was sufficient at particular times to foster survival. In many perennial grasslands achieving recruitment depends on several environmental variables being simultaneously favourable (O’Connor 1996). An understanding of the fluctuations in soil moisture throughout the rooting zone of perennial pastures would be necessary for an adequate understanding of the competitive environment faced by the seedlings as they attempt to compete with mature perennial grasses. This aspect was not explored as the experiments were restricted to the issues related to the initial recruitment of seedlings. One of the key variables identified was soil moisture levels generated from rainfall events in late summer. There was in general one major rainfall event in February that adequately recharged the soil moisture conditions for the recruitment to occur by early March. The recruitment events were associated with the availability of moisture in the top 50 mm of soil surface. This agrees with the view that the germination and emergence of grass seeds in the field requires adequate soil moisture (Wilson and Briske 1979; Maze *et al.* 1993; Hamilton *et al.* 1999; Zimmermann *et al.* 2008). The amount of rainfall and the actual rain days were not useful indicators of suitable soil moisture conditions.

The five recruitment events recorded across the five experiments occurred at the same time of the year, soon after seed set and with significant moist periods for several days. Differences between species (one C4 and two C3 grasses) were neither apparent in the timing of the recruitment event, nor in the general climatic conditions under which it occurred. Recruitment was recorded when the rainfall event (median 68 mm across the three sites) was predicted to keep the soil (0-50 mm) moist for at least 14 days with a maximum of 2 dry days in the period. This rainfall event occurred in the second half of February, sometimes extending to early March, when mature, germinable seed was present. This amount of February rainfall was just above the long-term average for that month at Trunkey Creek and Wellington, but less than the combined rainfall for February and March in all cases. Future research may show that the average February rainfall (62 and 60 mm at Trunkey Creek and Wellington respectively) is adequate for a recruitment event to occur. If the amount of rainfall is received outside the late February to early March period, the probability of recruitment arguably becomes lower, as no recruitment was recorded at other times in this series of experiments. These conditions set the boundaries to determine the frequency of achieving a recruitment event.

Across the sites, the rainfall events that recharged and maintained adequate soil moisture for about 15 days with not more than 2 dry days in the period had a higher probability of a recruitment event. These soil moisture conditions (*i.e*. 15 moist days) were usually generated from the rainfall events of around 70 mm occurring over 7 rain days in late February and in some instances extending to early March. The probability of exceeding the soil moisture conditions for 15 days (the number of moist soil days where all events observed resulted in reasonable levels of recruitment) in the February-March period was higher than initially expected. The worst case was that of Wellington where the probability of exceeding soil moisture conditions of 15 days was estimated at only 30% *i.e.* a chance of getting good recruitment of only once every 3 years. The other sites at Trunkey Creek and Orange had better probability estimates of 44 and 78%, respectively. The analyses of 30 years of climate data supported the experiment results that there is a respectable probability of adequate rainfall events, usually starting in February each year that would result in a recruitment event. Any average or above average years should result in recruitment in paddocks appropriately rested to maximise flowering and seed set and on appropriate soils where a light scarifying was done to increase the microsites for recruitment (Thapa 2010).

The time periods that had only 7 days of moist soil were considered to be the minimum below which reasonable recruitment was unlikely. This was based on the conditions generated from the irrigation experiments where recruitment was low. Attaining minimum conditions of soil moisture (*i.e.* 7 moist days) may mean there still is a possibility of little recruitment rather than a failure. The probability of exceeding 7 days of soil moisture was substantially higher and the estimates were between 72 and 98% across the field sites. This indicates there may be a chance of a small recruitment event in most years, if the conditions of seed and microsite availability are fulfilled. Recruitment can be enhanced by moderate soil disturbance (*e.g.* scarifying) and maximising the amount of available seed (Thapa 2010). Extending the climatic analysis to a number of sites across NSW, Victoria and Tasmania showed there were reasonable chances of achieving suitable moisture conditions for a recruitment event to occur. These results reinforce the view that suitable climatic conditions exist during late summer, early autumn across south-eastern Australia for a recruitment event to occur and therefore the principles developed would in general have wider regional application.

The surface soil (top 50 mm) was usually dry for a while after recruitment events in early March across the sites. These dry periods did not seem to adversely affect seedlings as significant numbers survived at 6 weeks after emergence. It may be that the radicle had already grown sufficiently to access water from soil below 50 mm. The average temperature for the period when the soil moisture was considered adequate during February-March was around 20˚C and that temperature is near optimal for germination of most of the perennial grass species (Baskin and Baskin 1998). This further substantiates the hypothesis that the rainfall events in late summer can be relied upon to generate adequate soil moisture and temperature conditions for recruitment events to occur in March.

The recruitment results from both the main field and irrigation experiments indicate that the important criterion for a recruitment event were the numbers of days the soil remained moist from a rainfall event rather than the amount of rainfall or the actual rain days.

Irrigation treatments did not achieve significant recruitment but provide a better understanding of the recruitment mechanisms. Swards were typical of most grassland in the district at the time. In these experiments water was applied over two days at a rate equivalent to a good rainfall event (50 mm) but the instances when irrigation did not coincide with actual rainfall failed to produce the soil moisture conditions required to achieve a recruitment event. This again highlights the importance of days of continuous soil moisture rather than rainfall *per se* as a key requirement for perennial grass recruitment. Irrigation does not generally simulate the atmospheric conditions that occur under natural rainfall, which allows moisture to remain in the soil surface for a longer period due to milder temperatures and higher absolute humidity (lower atmospheric vapour pressure deficit). The irrigation experiments support the hypothesis that subtle conditions at the soil surface are critical for seedling recruitment. There was however, some useful emergence within irrigation experiments at the same time as the main field experiments. In those instances viable seed was added to each plot to overcome any limitations in seed bank after adding water alone failed to significantly generate seedlings.

Rainfall events in late summer were useful for recruitment. This was in contrast to the ‘autumn break’ which is expected to occur later, but as these analyses show, this does not happen that often in central NSW. Over the last 30 years at these study sites reliable rainfall events started in February. No recruitment events were observed at other times of the year suggesting that the rainfall events that occurred in February through March are more reliable for recruitment as at other times of the year recruitment is limited by other factors (*e.g.* low availability of seed) even though reliable rainfall events occur.

The grasses in this study are known to exist throughout the south-east and do naturally recruit. This research suggests that throughout south-eastern Australia recruitment is probably occurring in late summer and autumn. Farmers are now in a position to more reliably enable recruitment of desirable perennial grasses within their pastures. The outcomes can be achieving 60% perennial grass at significantly lower-cost, at which improved production and environmental outcomes can be achieved.

Acknowledgements

The authors would like to thank Geoff Millar for modelling advice.

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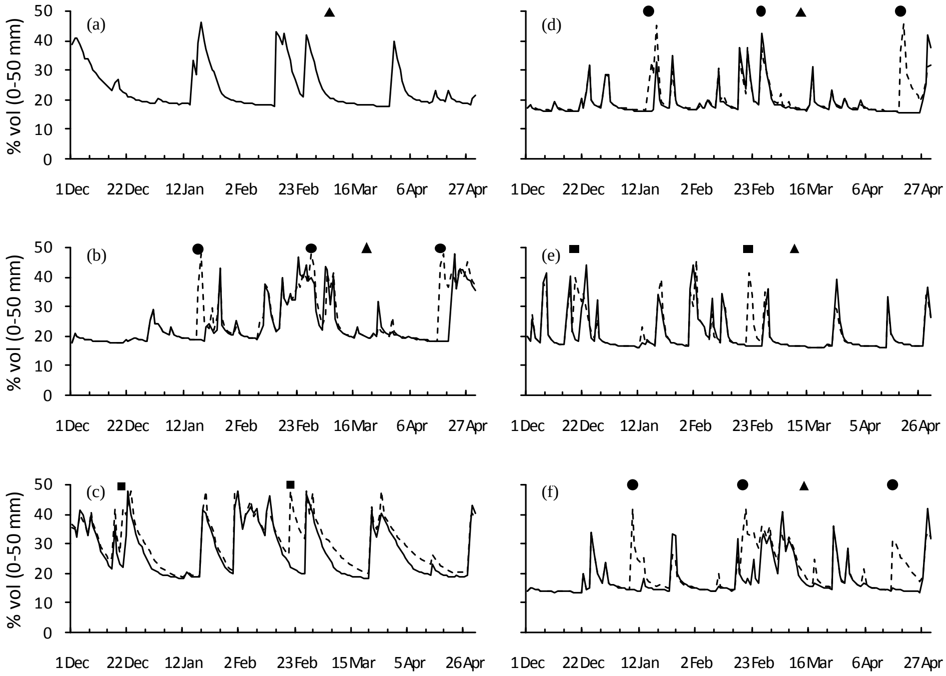
Fig. 1. Soil water content (% volume of soil) for the top 0-50 mm without irrigation (solid line) and with irrigation (dashed line) generated from Sustainable Grazing Systems pasture model from December to April at (a) *Phalaris aquatica* site in Orange, 2005-6, (b) *Phalaris aquatica* site in Orange, 2006-7, (c) *Phalaris aquatica* site in Orange, 2007-8, (d) *Austrodanthonia* spp. site in Trunkey Creek, 2006-7, (e) *Austrodanthonia* spp. site in Trunkey Creek, 2007-8, and (f) *Bothriochloa macra* site in Wellington, 2006-7; symbols represent different events (triangle = time of identified recruitment events in the main field experiments; circle = the time of irrigation treatment (50 mm over 2 days); square = seed addition in irrigation treatments).

Table 1. Median days of moisture for recruitment in the top 50 mm of soil and probability of exceeding the minimum and ideal soil moisture conditions of 7 and 15 days respectively and the average rainfall coinciding with the median, 7 and 15 days of moist soil over the last 30 years for February-March across the field sites and additional sites across NSW, Victoria and Tasmania; estimates are derived from individual probability distribution for each site (not presented) and quadratic relationships of rainfall versus moist soil days in Fig. 2; table arranged in latitude order to indicate the north south transect.

Fig. 2. Rainfall events in the February-March period and the corresponding estimated number of days of moist soil in the top 50 mm due to that particular event over the last 30 years across the field sites and additional sites across NSW, Victoria and Tasmania; quadratic curves fitted to the data to show trends.

Fig. 3. The first significant rainfall event after seed maturity starting from 1st February (denoted as rainfall start day 1) until 31st March (day 59) against the corresponding number of days of moist soil in the top 50 mm due to that particular event over the last 30 years across the field sites and additional sites across NSW, Victoria and Tasmania; quadratic curves fitted to show trends.


(a)
(b)
(c)
(d)
(e)
(f)
% vol (0-50 mm)
0
10
20
30
40
50
1 Dec
22 Dec
12 Jan
2 Feb
23 Feb
16 Mar
15 Mar
6 Apr
5 Apr
27 Apr
26 Apr

1 Fig. 1

Table 1

Site	Location (°S, °E)	Altitude (m)	Mean annual			Climatic conditions through February-March period for 30 years from 1975-2004								
										Probability (%) of exceeding		Estimated average rainfall (mm) at		
			rainfall (mm)	MaxT (°C)	MinT (°C)	Mean MaxT (°C)	Mean MinT (°C)	Median rainfall (mm)	Median days of moist soil	7 days of moist soil	15 days of moist soil	median days of moist soil	7 days of moist soil	15 days of moist soil
Armidale	30.52, 151.67	980	791	20.3	7.1	25.1	12.3	69.5	14.0	92	39	67	26	75
Tamworth	31.09, 150.85	404	673	24.3	10.2	30.1	15.9	50.3	10.0	76	5	56	39	84
Wellington	32.51, 148.97	300	618	22.8	10.5	28.9	16.2	49.6	10.5	73	30	48	29	79
Mudgee	32.60, 149.60	454	675	23.0	8.3	29.0	14.2	49.8	10.0	83	11	60	41	94
Orange	33.23, 149.12	826	890	17.6	7.2	23.8	10.9	52.1	20.0	98	78	72	20	48
Bathurst	33.43, 149.56	713	634	19.8	6.8	25.9	12.1	48.9	14.0	81	35	55	23	61
Trunkey Creek	33.82, 149.32	840	800	19.3	6.9	25.5	12.0	44.9	16.0	91	49	46	18	51
Goulburn	34.75, 149.70	670	638	19.7	7.4	25.3	12.4	48.0	11.0	85	9	58	33	91
Canberra	35.30, 149.20	578	615	19.7	6.5	25.8	11.9	39.6	9.5	84	5	64	34	97
Tallangatta	36.19, 147.36	220	833	21.4	9.9	28.7	15.1	39.2	8.0	61	8	30	25	89
Cooma	36.23, 149.12	778	530	19.4	4.1	25.1	9.3	42.7	9.0	83	8	33	23	58
Beechworth	36.37, 146.71	580	946	18.4	7.8	25.6	12.6	44.4	9.0	71	11	33	24	74
Warrenbayne	36.69, 145.88	225	853	18.5	6.0	25.7	10.3	28.3	10.0	62	18	35	23	63
Creighton's Creek	36.90, 145.52	276	841	18.5	6.0	25.7	10.3	30.4	8.0	56	0	29	23	49
Bombala	36.91, 149.24	705	641	18.4	4.8	23.8	9.5	47.7	11.0	87	26	47	27	73
Mansfield	37.05, 146.09	316	719	20.9	5.1	27.3	11.5	33.5	8.5	67	3	34	28	60
Highlands	37.07, 145.42	565	852	20.3	7.8	27.7	13.2	23.6	9.0	66	0	28	19	40
Tambo Crossing	37.51, 147.85	195	767	18.9	9.9	23.4	13.4	46.7	13.0	79	22	53	24	68
Launceston	41.54, 147.20	166	676	17.0	6.2	22.2	9.6	26.7	8.0	64	8	22	19	50
Cressy	41.72, 147.08	148	628	17.2	5.1	22.5	8.7	22.4	10.0	64	18	30	19	51
Avoca	41.78, 147.72	205	551	17.7	5.2	22.2	8.9	24.5	9.0	56	13	33	26	55
Swansea	42.12, 148.07	6	595	17.9	7.8	21.6	11.1	26.3	10.0	83	15	28	18	37
Hobart	42.89, 147.33	51	616	16.9	8.3	20.9	11.5	27.3	10.5	65	13	29	18	50
Dunalley	42.90, 147.87	12	697	17.5	8.0	21.5	11.4	40.7	11.0	83	26	29	17	45

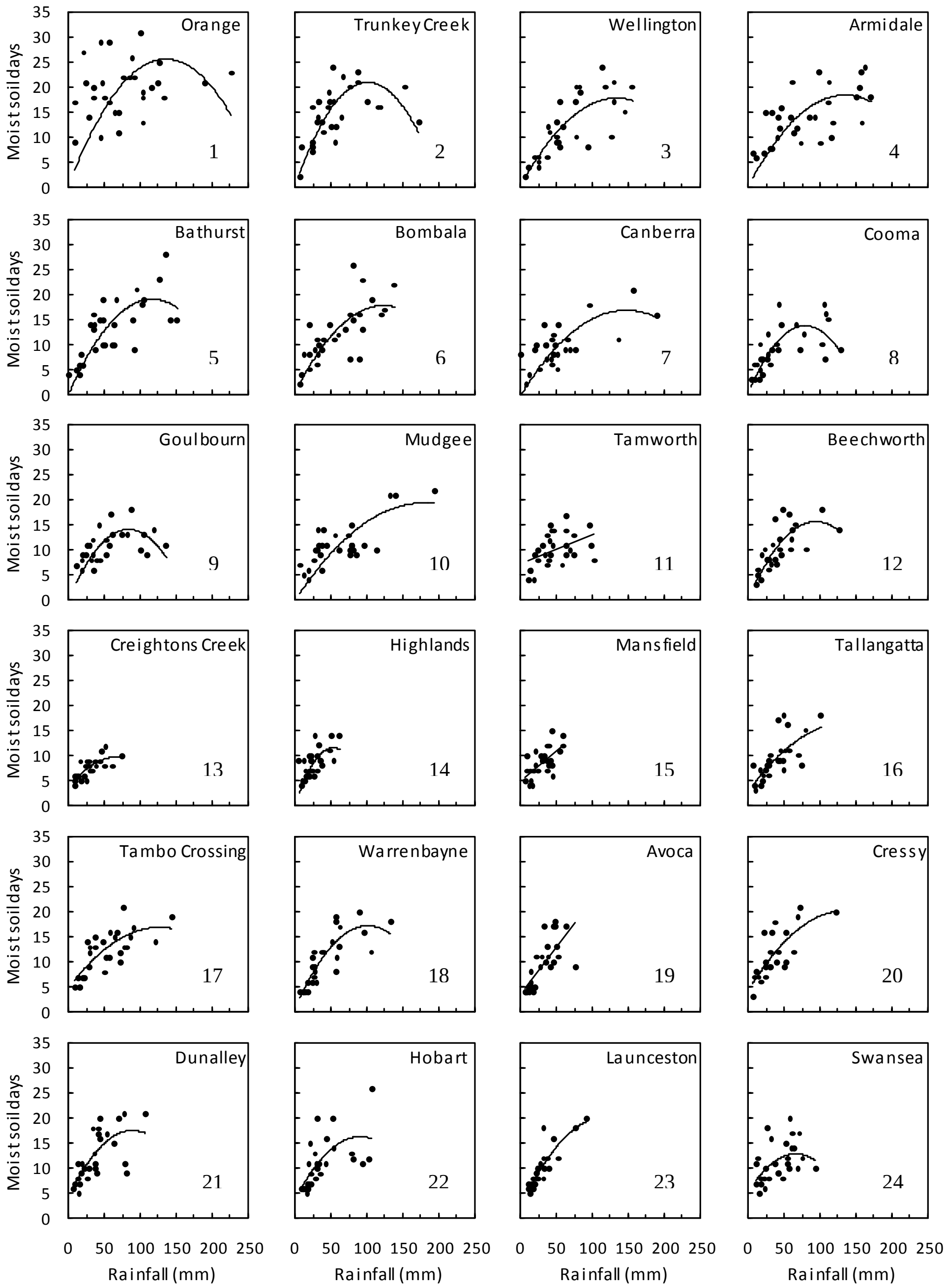
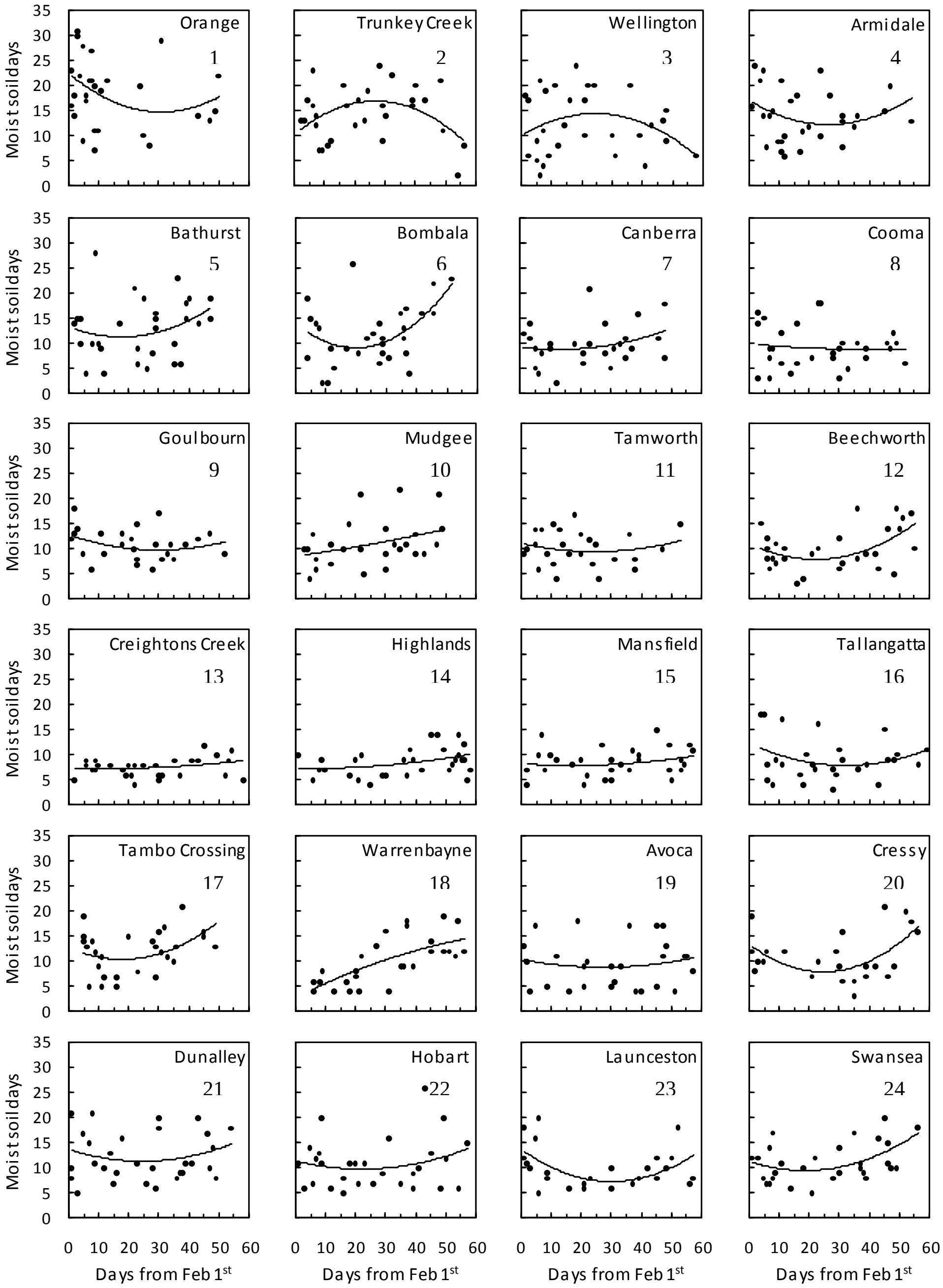

Moist soil days
Rainfall (mm)
0
5
10
15
20
25
30
35
0 50 100 150 200 250
Orange 1
Trunkey Creek 2
Wellington 3
Armidale 4
Bathurst 5
Bombala 6
Canberra 7
Cooma 8
Goulbourn 9
Mudgee 10
Tamworth 11
Beechworth 12
Creightons Creek 13
Highlands 14
Mansfield 15
Tallangatta 16
Tambo Crossing 17
Warrenbayne 18
Avoca 19
Cressy 20
Dunalley 21
Hobart 22
Launceston 23
Swansea 24

Fig. 2


Orange 1
Trunkey Creek 2
Wellington 3
Armidale 4
Bathurst 5
Bombala 6
Canberra 7
Cooma 8
Goulbourn 9
Mudgee 10
Tamworth 11
Beechworth 12
Creightons Creek 13
Highlands 14
Mansfield 15
Tallangatta 16
Tambo Crossing 17
Warrenbayne 18
Avoca 19
Cressy 20
Dunalley 21
Hobart 22
Launceston 23
Swansea 24
Moist soil days
0 5 10 15 20 25 30 35
0 10 20 30 40 50 60
Days from Feb 1st

1 Fig. 3

Recruitment of *Phalaris aquatica* within existing swards 1. Effects of biomass manipulation, seed level modification and site preparation

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Short title: Recruitment of *Phalaris aquatica* seedlings

Abstract. A successful recruitment event in perennial grasslands is infrequent and when it occurs, the rate of recruitment and survival is low, less than 1% in most occasions. This paper reports on two field experiments that investigated the effects of biomass manipulation, seed level modification and site preparation on the recruitment of *Phalaris aquatica* seedlings. The experiments were done through drier than average years, where *P. aquatica* achieved successful recruitment of seedlings. Recruitment rates proportional to total seed set were low (2.9% in Experiment 1 and 0.4% in Experiment 2). The control treatment resulted in 352 seedlings m^{-2} in Experiment 1 and 16 m^{-2} in Experiment 2 compared to the best treatments which had 500 m^{-2} and 38 m^{-2} respectively. Low emergence in Experiment 2 reflected the poor seed set before the recruitment event. Seed addition had little effect on recruitment suggesting that soil seed reserve was not limiting in the system. Soil scarification failed to have any significant effects in both experiments. Seedlings survived until the following summer, but few then remained through the ensuing drought.

Additional keywords: seedling recruitment, seedling survival, seed set, soil scarification, insecticide, seed addition

Introduction

Phalaris aquatica L. is a temperate (C3) perennial grass species that has been widely sown in the Australian environment since it was introduced in 1884 from Europe via the United States (Watson *et al.* 2000). *P. aquatica*, a native of southern Europe, North West Africa and the Mediterranean region, is one of the more persistent and productive temperate perennial pasture grasses. The usefulness of *P. aquatica* as a permanent pasture species has been recognised and well demonstrated in the temperate regions of Australia (Watson *et al.* 2000; Lamp *et al.* 2001). The species is drought tolerant, can withstand extended periods of heavy grazing, performs well in poorly drained and waterlogged soils, and provides good quality forage for all types of grazing livestock. It is known to grow successfully on a wide range of soil types, from heavy soils to sandy soils, although it is not very tolerant of soils with high aluminium, acidity and low fertility (Lamp *et al.* 2001). A number of cultivars have now been bred for Australian conditions and these are more tolerant of low soil pH.

P. aquatica produces large quantities of seed. Kelman and Culvenor (2007) observed 2000 to 11000 seeds m^{-2} dispersed, with an average of 4600 m^{-2} over 3 years of study at a field site near Canberra, Australia. In ungrazed *P. aquatica* grasslands in Spain, up to 25000 seeds m^{-2} have been recorded (Leiva and Alés 2000). However, little recruitment within *P. aquatica* stands has been reported (Dowling *et al.* 1996; Virgona and Bowcher 2000; Lodge 2004). Kelman and Culvenor (2007) reported recruitment was around 0.1% of the total seed rain in any one year during 3 years of an experiment.

The most often advanced reason for low *P. aquatica* recruitment is competition from annual grasses (Virgona and Bowcher 1998; Lodge 2000) and sown legumes (McWilliam *et al.* 1970; Lamp *et al.* 2001), which prevent the weak *P. aquatica* seedlings from establishing. High levels of seed harvesting by ants is an additional factor that contributes to low recruitment in *P. aquatica* swards (Campbell 1966; Campbell and Gilmour 1979; Kelman *et al.* 2002). *P. aquatica* recruitment is further limited by the ability of adult plants to colonise new spaces (Campbell *et al.* 1981; Lodge 2004). In contrast, *P. aquatica* spreads readily by seedling recruitment along ungrazed roadsides in south-eastern Australia (Kelman *et al.* 2002; Lodge 2004). Recruitment obviously occurs at times under natural conditions, but the mechanisms are poorly understood.

To achieve recruitment of new plants several stages need to occur. Once the current seed is mature and ready to germinate, it then needs to reach the ground to establish new plants. Seeds can remain within seed heads above ground and drop gradually to the ground. Once on the ground surface seeds can be taken by predators such as seed harvesting ants. This could mean only a small quantity of seed is available for potential germination at one time. As seeds drop, the ground surface acts as a potential site for germination. The ground could be bare, covered with litter, interspersed with rocks and twigs, intersected by fissures or simply occupied by existing mature plants. Apart from the soil surface features there are likely to be underground roots of existing perennial plants already exploiting the area and competing for the below ground resources of soil, water and nutrients. The aim of experiments reported in this paper was to investigate mechanisms on how best to deliver the seed to the soil surface (maximising the number of germinable seeds that reach the ground at one time) and how the sites at the soil surface can be modified (through disturbance and biomass manipulation) and microsites created to improve the chance that seeds will successfully germinate and seedling establish.

Methods

Site

The experiment was located at Orange on the Central Tablelands of New South Wales (149°07' E, 33°14' S) within the Orange campus of Charles Sturt University. The site had an elevation of 826 m (a.s.l.) and an average annual rainfall of 890 mm. There was a slight easterly slope on the site. Prior to the experiment the paddock had been rotationally grazed by cattle and sheep, with longer rest than graze periods. The site had a history of fertiliser application and had been sown with introduced pasture species many years before. The soil type was a Vertosol (Isbell 1996) which was a very dark grey colour (Oyama and Takehara 1970) with a light clay surface texture (McDonald and Isbell 1990), and a pH of 5.2 ($CaCl_2$) and 5.9 (water). The experiment was established in October 2005 within an existing *P. aquatica* dominant pasture (40 plants m^{-2}). Annual grasses were limited to a few species (*Bromus* spp., *Lolium rigidum* Gaud., *Poa annua* L., *Vulpia* spp.). *Dactylis glomerata* L., a sown C3 perennial grass, was present. There were limited weed problems and the main competitor for emerging *P. aquatica* seedlings was mature *P. aquatica* plants. *P. aquatica* is a very competitive plant and this site was chosen to provide a moderately to highly competitive environment. The field research started when the *P. aquatica* seed heads were starting to emerge. The site was fenced off to exclude grazing throughout the whole study.

Climate data measured at the site included: soil temperature, soil moisture (0-50 mm), relative humidity, air temperature, solar radiation, wind speed and rainfall. Weather was recorded using an automatic weather station (Tain Electronics®). The monthly rainfall and average temperature (minimum and maximum) recorded for the duration of the experiment are summarised in Fig. 1, along with the long-term averages (1968-2007) measured at an official Australian Government Bureau of Meteorology site located within 15 km of the experimental site (BOM 2008). Rainfall was below the long-term average in most months during the experiment and 2006 was an extremely dry year with only 304 mm of annual rainfall. The mean annual maximum daily temperature was 17.6°C with monthly means ranging from 25.9°C in January to 9.3°C in July. The mean annual minimum daily temperature was 6.1°C with monthly means ranging from 12.1°C in January to 0.7°C in July. Average maximum daily temperature was higher and minimum daily temperature was lower than the long-term averages in most months during the experiment.

[Fig. 1]

Experimental design

The experimental design was based upon a factorial combination of 3 seed delivery mechanism treatments x 3 seed treatments x 3 site preparation treatments laid out in a randomised block with 4 replicates. The experiment was repeated over two years (Experiments 1 and 2) on separate plots to capture the establishment in two different years; Experiment 2 (second year) being a repeat of Experiment 1 (first year) on an adjoining site. All plots in Experiment 1 were within a single block of 108 plots whereas plots in Experiment 2 were split into 2 blocks each with 2 replicates due to an area constraint. Treatments were applied at the start of summer in January 2006 for Experiment 1 and in January 2007 for Experiment 2.

Treatments

The seed delivery mechanism treatments were applied when it was considered seeds were reaching maturity but before seed fall. They varied the level of mechanical disturbance, litter levels, plant cover and structure and delivery mechanism of mature seed to the soil surface. The 3 seed delivery mechanism treatments were: uncut or control (UC), cut and leave (CL), and cut and remove (CR). In UC, the sward was left uncut with seed heads standing to follow the natural cycle. The CL had standing plant material cut to a height of 20-50 mm above ground but plant material and seed heads were left on the ground. This aimed to increase the litter level as a means to retain more moisture near the soil surface and also to maximise the number of seeds on the ground at one time. It was postulated that a large amount of seed reaching the ground at once could limit the ability of seed harvesting ants to completely remove them and enable some seeds to germinate. In CR, herbage mass was reduced to a level similar to grazing by cutting to a height of 20-50 mm above ground and plant matter removed. This treatment aimed to simulate the physical movement from grazing that would have caused some seeds to drop to the ground, while others could be consumed through grazing.

The seed treatments were designed to determine if current seed rain would maximise seedling numbers, if it was limiting, or if seed predation was a problem. Seed harvesting ants can be a major problem with some grass species, *P. aquatica* in particular. There were 3 seed treatments: no seed addition or control (NS), insecticide application (IS) and seed addition (SA). In IS, dead seeds (~50 kg ha^{-1}) treated with Gaucho® (a.i. 600 g L^{-1} imidacloprid) were added to limit predation from ants on existing viable seeds. These insecticide treated seeds were tested for germination (no germination recorded) before field application. *P. aquatica* seeds were used as they are very attractive to ants (Campbell 1966; Campbell and Gilmour 1979). For SA, extra *P. aquatica* seeds were added (~50 kg ha^{-1}) to test if current seed rain had saturated the system and if recruitment was limited by seed availability. The aim was to flood the system with additional seeds. These seeds were tested for germination (85% at 15°C; 87% at 25°C; 68% at 35°C) before field application. A response to this treatment would also indicate that there was no limitation of microsites for seedling recruitment.

The site preparation treatments were designed to modify the potential sites for seedling recruitment by modifying the soil surface layer and by reducing competition for emerging seedlings through herbicide application. The 3 treatments included: no preparation or control (NP), herbicide application (HP), and scarify and rake (SR). The plot was left unmodified in NP. In HA, a sub-lethal dose of Fusilade® (a.i.: 212 g L^{-1} FLUAZIFOP-P present as the butyl ester) at a rate of 250 mL ha^{-1} was applied (25 January 2006 in Experiment 1; 7 February 2007 in Experiment 2) to kill any annual grasses that were germinating prior to *P. aquatica* germination events while limiting damage to the existing mature plants. This treatment was applied before any seed addition. For SR, the ground surface was scarified to remove small competitors and existing adult plants. Approximately 50% of the plot area was scarified uniformly to create more bare ground and roughen soil surfaces which could become potential microsites for seedling recruitment. This treatment aimed to create microsites that may be favourable for germination under suitable climatic conditions. This treatment also changed the location and density of litter biomass.

Plot layout

Plots were 2 x 2 m. Treatments were applied over the whole 2 x 2 m plot. Each plot had 3 levels of measurement in the layout: 0.9 x 0.9 m, 0.3 x 0.3 m and 0.1 x 0.1 m. The centre of the plot was 0.9 x 0.9 m and permanently marked for routine measurements. Outside of the 0.9 x 0.9 m measurement area was a buffer between the adjoining plots that was used to collect additional measurements (*e.g.* soil seed bank and soil samples). The 0.9 x 0.9 m area was subdivided into nine 0.3 x 0.3 m contiguous quadrats (arranged in a 3 x 3 square) and used to measure biomass and plant species composition. Each 0.3 x 0.3 m quadrat was further divided into 81 0.1 x 0.1 m sub-quadrats arranged within the 0.9 x 0.9 m permanently marked area. Seedling numbers were recorded in these sub-quadrats. The total area for seedling measurement overlaid the same area as the quadrats used for biomass and plant composition estimates. The seedling numbers were combined for the 9 sub-quadrats (0.1 x 0.1 m each) overlaying the 0.3 x 0.3 m quadrat for comparisons at that level.

Measurements

Dry weight ranks of the 3 most abundant species and the total dry matter (DM) of all species were estimated using BOTANAL procedures (Tothill *et al.* 1992). Ranked species were combined into plant functional groups using a subjective method defined by Gitay and Noble (1997) which is based on a combination of life history, physiological and abundance characteristics. Dry weights of standing DM (t ha^{-1}) and litter DM (t ha^{-1}) were estimated separately and the estimates were corrected using 15 to 20 calibration cuts at each sample period (Sanford *et al.* 1998). Sampling for pasture biomass was made every 3 months in the late summer (February), autumn (May), winter (August) and spring (November) of each experimental year. Plant cover, litter cover and bare ground percentages were visually estimated (Sanford *et al.* 1998) and ratings were in 5% increments (*e.g.* 0, 5, 10 to 100). Plant cover was defined as the area on the ground covered by standing biomass when projected vertically on to the soil surface. Litter cover represented the portion of the ground surface covered by detached and dead material excluding the basal area of the standing plants. Bare ground corresponded to the area which was bare in terms of soil exposure plus the area covered by any non-plant material (*e.g.* cow manure, rocks, tree branches) present.

Sampling frequency for seedling monitoring varied with time from germination events. The emergence immediately following treatment application and a subsequent substantial rainfall event was the major germination event to be monitored through the year. Survival was defined as the young plants that lived through the first summer. Initial counts were done within 2-3 weeks of seeds germinating (after treatment application); then approximately after 6, 24 and 52 weeks. Each sample counted all the seedlings present and marked those newly emerged with coloured nails for the monitoring of young plant survival.

Soil cores were taken across the site to determine the seed bank. The cores were taken at the start of experiment (after treatment application) in both experiments. Samples were only taken in those treatments where natural seed fall was assumed to differ, so the treatments that had addition of extra seeds were avoided. Within the plot, 2 soil cores (0.05 m diameter x 0.05 m deep) were randomly collected - one from each side of the 0.9 x 0.9 m area used for measurements. The cores for each plot were mixed, brought to the glasshouse, sifted, added to the surface of sand and placed into regularly watered pots. All seedlings that emerged were identified, counted, recorded and then combined into functional groups. The experiment continued until there were no more seedlings emerging, usually for a period of 2 weeks. An aim was to determine the readily germinable species that could compete with the emerging perennial grass seedlings.

Seed production across the site was estimated through each flowering season, which occurred over the summer. The total number of plants and seed heads in each of those plants were counted in 10 randomly selected 0.3 x 0.3 m quadrats across the site at the time of seed maturity. From these plants 10 seed heads were collected and seeds were counted to estimate the amount of seed per seed head and total seed production. These measurements were made prior to the implementation of any treatments.

P. aquatica seedlings were first observed during early autumn (March) after a substantial rainfall event. The final count was made in the following autumn after the next summer following the initial germination event. In Experiment 1 (2006-7) initial observations for seedlings were made on 7 March 2006 followed by young plant survival counts on 26 April 2006, 25 August 2006 and the final count on 19 March 2007.

Experiment 2 (2007-8) followed a similar sequence with seedling counts on 21 March 2007, 23 April 2007, 31 August 2007 and 4 March 2008.

Analyses

Differences between treatments in total standing biomass, green and litter biomass, each functional group biomass, bare ground and plant cover at each measurement period were analysed by Restricted Maximum Likelihood (REML) spatial analysis to ensure spatial variability was accounted for. A regular grid with AR1 (first-order autoregressive model) was used for Experiment 1 and an irregular grid with a power model and Euclidean distance measure was used for Experiment 2 due to the arrangement of plots. Significance was determined through Wald tests.

Due to data being non-normally distributed, a Generalised Linear Mixed Model (GLMM) analysis assuming Poisson distribution with a logarithmic link function was used to determine differences in seedling numbers between treatments at each measurement period. Statistical significance was determined through Wald tests. Across sites there were often trends in seedling recruitment that were a site effect, rather than of treatments. Spatial analysis techniques (REML) were able to resolve the underlying treatment effects.

All statistical analyses were done using GenStat 9.1® (Payne *et al.* 2006).

Results

Treatments were designed to create variation in the vegetation structure of the sward, viable seed levels and suitable microsites for germination. Since the subset of treatments in modifying seed levels would not affect the sward structure, the results (and graphs) on grassland description and species composition are presented only for the subset of treatments in seed delivery mechanism and site preparation. Results (and graphs) on seedling recruitment and survival are presented across all subsets of treatments as modifying seed levels represents one of the most significant factors for emergence of seedlings.

Grassland description

Experiment 1 (2006-7)

UC had about twice the standing DM of CL or CR (P<0.001) and no significant difference occurred between CL and CR throughout Experiment 1 (Fig. 2-a). SR removed the bulk of the herbage mass resulting in lower standing DM than NP or HA (P<0.001). HA did not significantly reduce standing DM compared to NP but this was expected because it was primarily designed to kill any germinating annual grasses rather than to modify the vegetation structure. Immediately after treatment application in late summer 2006, the average standing DM was 3.4 ± 0.2 t ha^{-1} (UC = 5.3 ± 0.2 t ha^{-1}; cut = 2.5 ± 0.2 t ha^{-1}) which increased to 4.0 ± 0.1 t ha^{-1} by late autumn 2006 but declined afterwards until the end of Experiment 1 in late summer 2007 to 1.5 ± 0.1 t ha^{-1} which reflected the adverse effects of a dry year.

Green DM was positively correlated with total standing DM across all treatments in Experiment 1 (*data not presented*). More green DM was observed where more standing DM was present (*i.e.* in UC) and followed similar declining trends as the standing DM (Fig. 2-b). CL or CR had around half the green DM compared to UC (P<0.001), a trend which remained throughout until a year later in late summer 2007 when green DM was about the same (~0.4 t ha^{-1}) across all treatments. Similarly, SR had approximately half green DM compared to NP or HA (P<0.001) but the difference was bridged by autumn 2006 and then had similar growth rates until the end of Experiment 1 in late summer 2007. Green DM was highest during late autumn 2006 (0.9 ± 0.1 t ha^{-1}) across all treatments except under UC x HA (highest at the start of Experiment 1 in late summer 2006).

Though litter DM fluctuated between seasons, a declining trend across all treatments was evident as Experiment 1 progressed (Fig. 2-c). Differences were apparent at the beginning of Experiment 1 in late summer 2006 (more litter DM in CL than UC or CR, P<0.001; less in SR than NP or HA, P<0.001) but did not remain throughout Experiment 1. By autumn 2006 litter DM was similar for all treatments (~2.6 t ha^{-1}) and remained that way until the end in late summer 2007. Litter DM decreased from the start of Experiment 1 (3.2 ± 0.1 t ha^{-1}) until spring 2006 (1.5 ± 0.1 t ha^{-1}) but increased slightly the following year in late summer 2007 (1.8 ± 0.1 t ha^{-1}).

On average 62 ± 1% bare ground was created in SR when there was less than 5% naturally occurring bare soil surface in late summer 2006 (Fig. 2-d). UC had less bare ground than CL or CR (P<0.001) and SR had more compared to NP or HA (P<0.001). Across all treatments, more space for germination was available in CR x SR (P<0.001). The percentage of bare ground gradually decreased throughout the year and there was hardly any bare ground observed from winter 2006 to the end of Experiment 1 in late summer 2007 (P<0.001).

Plant cover at the start in late summer 2006 was greater in UC than CL or CR (P<0.001), and this difference remained throughout Experiment 1 except in autumn 2006 (Fig. 2-e). Though less plant cover was observed in SR than NP or HA (P<0.001) at the start of Experiment 1, the levels were similar from autumn 2006 till the end of Experiment 1 in late summer 2007. The percentage of plant cover gradually increased throughout the experiment from 21 ± 3% (late summer 2006) to 56 ± 2% (late summer 2007).

[Fig. 2]

Experiment 2 (2007-8)

Drought conditions in 2006 resulted in generally lower levels of standing DM at the start of Experiment 2, which then increased during the next 52 weeks to similar values as those in Experiment 1 (Fig. 3-a). The trends in Experiment 2 were similar to Experiment 1 in that UC had twice as much standing DM as CL or CR ($P<0.001$), SR had less ($P<0.001$) and there was no significant difference between CL and CR. For the whole of Experiment 2, UC x NP had the most standing DM ($P<0.001$) and CR x SR the least ($P<0.05$) except in the end (late summer 2008) least standing DM occurred in CL x HA ($P<0.001$). For UC average standing DM at the start of Experiment 2 in summer 2007 was 1.7 ± 0.1 t ha^{-1} whereas cut treatments had 0.8 ± 0.2 t ha^{-1}; this was about one-third of that at the start of Experiment 1 and similar to the levels at the end of Experiment 1. There was a gradual increase until spring 2007 (4.1 ± 0.2 t ha^{-1} in UC; 3.3 ± 0.2 t ha^{-1} in cut treatments) with slow growth during autumn and winter but declined slightly the following year in late summer 2008 (3.5 ± 0.2 t ha^{-1}), when Experiment 2 ended *i.e.* only marginally less than the year before. The major difference between the two experiments was that in Experiment 1 standing DM declined throughout the experiment while the reverse applied in Experiment 2.

Similar to Experiment 1, a positive correlation of green DM with standing DM was observed across all treatments in Experiment 2 at least for the first 24 weeks from germination (*data not presented*). More green DM was present in UC ($P<0.001$) and less in SR ($P<0.001$) (Fig. 3-b). In general, green DM increased (compared to the reverse that applied in Experiment 1) except from spring 2007 to summer 2008 when green DM decreased slightly; the margin (~0.1 t ha^{-1}) of decline was similar to the increment for the same period in Experiment 1. Initial average green DM was low at 0.34 ± 0.04 tha^{-1} (approximately half of Experiment 1) then became highest during spring 2007 (1.5 ± 0.1 t ha^{-1}) before declining slightly at the end of Experiment 2 in late summer 2008 (1.4 ± 0.1 t ha^{-1}). These were lower initial and higher final values than in Experiment 1.

Litter DM in Experiment 2 declined in a similar manner to Experiment 1 (Fig. 3-c). At the start of Experiment 2 in late summer 2007 CL had understandably, more litter DM than UC or CR ($P<0.001$), and SR had less than NP or HA ($P<0.001$). By autumn 2007 through to the end of Experiment 2, the litter DM levels were more or less similar across all treatments. Initially, the site had 1.6 ± 0.1 t ha^{-1} litter DM (similar levels to when Experiment 1 ended) which decreased until the end of Experiment 2 (0.83 ± 0.03 t ha^{-1}), except in CR and SR where litter DM increased in autumn 2007 but declined afterwards. Compared to Experiment 1, both the initial and final values were lower.

There was a very low percentage (~5%) of natural bare ground available across the site in Experiment 2 but SR created $60 \pm 1\%$ bare ground, which was marginally less than Experiment 1 (Fig. 3-d). CR x SR had the most bare ground whereas UC x NP / HA and CL x NP did not have any bare patches ($P<0.001$). Similar to Experiment 1, the percentage of bare ground declined and was nil (total cover) by winter 2007 through to the end of Experiment 2 in late summer 2008. The rate of decline was similar in both experiments.

The changes in plant cover in Experiment 2 were similar to Experiment 1 (Fig. 3-e) and showed an overall increasing trend. UC always had more plant cover than CL or CR ($P<0.001$). Less plant cover was always present in SR than NP or HA ($P<0.001$) except during spring 2007 and the following year in late summer 2008 when no difference occurred. For the whole of Experiment 2, plant cover increased ($P<0.001$) gradually from the start in late summer 2007 ($16\pm3\%$) until spring 2007 ($77 \pm 2\%$) but decreased ($P<0.001$) slightly at the end ($72 \pm 3\%$). The initial values were a little lower but the final values were significantly higher than in Experiment 1.

[Fig. 3]

Species composition

Even though the site was dominated by *P. aquatica* a total of 25 species were recorded during the course of the two experiments. As *P. aquatica* was the bulk of the total pasture composition, changes in its DM (Fig. 4) followed similar patterns to total standing DM in both experiments (Fig. 2-a, 3-a). The functional groups formed for the analysis of botanical composition were *P. aquatica* (P), other C3 perennial grasses (C3), annual grasses (AG) and other species including forbs, legumes and sedges (OTH). Species such as forbs, legumes, sedges, broadleaves which ranked low in BOTANAL were combined into a single functional group (OTH) otherwise there would have been very low and, or missing values limiting the analyses possible.

[Fig. 4]

Experiment 1 (2006-7)

P. aquatica dominated the species mix throughout Experiment 1 (Fig. 4-a,b,c), accounting for >80% of the total DM in all seasons. UC had more than double *P. aquatica* DM compared to CL or CR ($P<0.001$) whereas SR had less than NP or HA ($P<0.001$) throughout Experiment 1. UC had 4.7 ± 0.3 t ha^{-1} *P. aquatica* DM in summer 2006 while cut treatments had less than half (2.1 ± 0.3 t ha^{-1}), which by autumn increased across most of the treatments (UC = 5.7 ± 0.3 t ha^{-1}; cut = 2.4 ± 0.3 t ha^{-1}) before declining until the end of Experiment 1 (UC = 2.3 ± 0.2 t ha^{-1}; cut = 0.8 ± 0.2 t ha^{-1}). Presence of AG (2-4%), C3 species (3-12%) and others (3-8%) were low during Experiment 1 and their proportions only changed marginally as the year progressed. On average, each functional group (other than *P. aquatica*) had less than 1 t ha^{-1} DM throughout Experiment 1 (Fig. 4-a,b,c).

Experiment 2 (2007-8)

P. aquatica and other members of the C3 perennial grass functional group (mainly

D. glomerata) dominated the species mix in Experiment 2 (Fig. 4-d,e,f). The total biomass consisted of ~60% *P. aquatica* and 30% other C3 species. The proportion of the C3 functional group (other than *P. aquatica*) in Experiment 2 was substantially higher than Experiment 1. There was less than 2% AG and 8% other species including broadleaf weeds; these proportions were similar to Experiment 1. After treatments commenced in summer 2007, there was 1.5 ± 0.3 t ha^{-1} *P. aquatica* DM in UC and 0.5 ± 0.3 t ha^{-1} in cut treatments, which increased across all treatments by autumn (UC = 1.9 ± 0.5 t ha^{-1}; cut = 1.0 ± 0.5 t ha^{-1}); this growth rate was similar to that of Experiment 1. During winter there was very little growth but *P. aquatica* added around 1 tonne of herbage in spring (UC = 2.7 ± 0.8 t ha^{-1}; cut = 2.0 ± 0.8 t ha^{-1}) before decreasing slightly at the end of Experiment 2 in summer 2008. As the seasons progressed, biomass of all functional groups increased across all treatments and there was more biomass at the end of Experiment 2 than it was in the beginning. This was in contrast to Experiment 1 where there was less biomass at the end than at the start (*i.e.* growth of all functional groups in Experiment 1 declined while the reverse applied in Experiment 2).

Recruitment patterns

Flowering and seed set

Flowering started in the second week of December continuing until mid January *i.e.* approximately 4 weeks, in both experiments. Anthesis commenced at the tip of the seed head, progressing towards the base over a relatively short period of 2-3 days. Seed retention in the head was greatest soon after anthesis, but then over 3-4 weeks seeds were released. Seed production was maximum during the month of January, declining over time but some seeds were still present in standing seed heads until May.

Seed production was lowest (57 kg ha^{-1}) towards the end of Experiment 1, which coincided with the start of Experiment 2 (Table 1). Seed weight (~1 mg per seed) remained relatively constant for all the seeds recovered from seed heads throughout summer and autumn. Germination of collected seeds over the summer averaged between 40-50% at the optimal temperature of 25°C. Seeds were tested for germination immediately (1-2 weeks) after collection.

[Table 1]

[Table 2]

Seed availability

Low quantities of *P. aquatica* seeds were present in the 'readily germinable' soil seed bank in both experiments (Table 1) accounting for only 2 and 3% respectively of the total germinating seed bank. Soil cores were taken during seed maturation hence these seeds were presumably from the current seed set. The low numbers were less than the seedling numbers for the best treatment in Experiment 1. AG were low in numbers compared to seasonal broadleaf species or legumes, which dominated the seed bank. The high numbers of legume seedlings were unexpected given that few legume plants were observed in either experiment. This suggests that most of the legume seed was softened by conditions used in the seed bank testing, but they may have remained hard in the field.

The germination rate of freshly fallen seeds was less than 50% but the amount of viable seed from the natural seeding was still large, 59 kg ha^{-1} in Experiment 1 and 24 kg ha^{-1} in Experiment 2 (Table 2) indicating that recruitment of *P. aquatica* in this pasture may not be limited by availability of seed. Across the experimental site seed addition treatment did result in more seedling recruitment on average but the proportional gain was only 2.5 and 0.7% in Experiments 1 and 2 respectively (Table 2). Natural seeding resulted in 2.9% of seedlings (165 m^{-2}) recruiting in Experiment 1 (Table 2) when seed yield was also high. The best treatment without added seed in both experiments had markedly higher emergence than seed addition, indicating extra seed only added to what happened naturally (Table 2).

General recruitment, overall young plant survival and treatment effects

Experiment 1 (2006-7)

In Experiment 1 recruitment of *P. aquatica* seedlings occurred in early March 2006 (Fig. 5-i) after rainfall events in the preceding months (Feb: 57 mm; Jan: 39 mm; Fig. 1). As many as 1300 seedlings m^{-2} were recorded in UC x IS x NP. Recruitment of seedlings occurred in all treatments. *P. aquatica* seedlings first germinated, UC had more seedlings than CL, which had marginally higher seedling numbers than CR ($P<0.001$) (Fig. 5-i). Fewer seedlings germinated in NS than IS or SA ($P<0.001$). IS and SA did not differ significantly but more seedlings were observed in IS than SA when herbicide was applied ($P<0.05$). Seedling recruitment was marginally more in HA than in NP or SR ($P<0.05$). No significant difference occurred between NP and SR except under CL when less seedlings were observed in SR than NP ($P<0.05$). Within UC, both IS and SA increased seedling number in SR; within CR, IS had more seedlings germinated in HA whereas there were more in NP for SA; and within CL, IS enhanced seedlings in NP but SA had more in HA or SR ($P<0.001$) (Fig. 6-i). Almost half the plots (46%) had more than 100 seedlings m^{-2}, but 3 plots (CL x IS x HA, CR x NS x NP and CR x IS x HA) did not record any seedlings. The site had received a substantial amount of seed set (Table 1) before any recruitment was observed. Seedlings of species other than *P. aquatica* were present but in low numbers across all treatments. The most prominent was legumes (~20 seedlings m^{-2}). Broadleaf seedlings were minimal (~1 m^{-2}) and no AG seedling was observed. Perennial grass seedlings (mainly *D. glomerata*) were around ~10 m^{-2}. These seedlings did not directly relate to treatments except for legumes which occurred more in UC than cut treatments

($P<0.05$).

Survival rates for young *P. aquatica* plants were very low (Fig. 5-ii,iii). During Experiment 1 there was less than average rainfall for the whole year. On average, 222 seedlings m^{-2} germinated at the site but there was a massive mortality throughout the year. The really important period of mortality was during the period from early March (Fig. 5-i) to mid autumn (Fig. 5-ii). Over this 6 week period more than 90% of emerged seedlings died in all treatments. Young plant survival across treatments followed similar trends to initial seedling numbers and UC still had higher seedling numbers than either CL or CR ($P<0.001$) (Fig. 5-ii). IS or SA had more seedlings surviving than NS ($P<0.001$) but equal numbers of seedlings were surviving in NP, HA or SR. All the significant interactions observed during emergence across treatments were present at this stage for surviving seedlings (Fig. 6-ii). At 24 weeks after emergence (early spring), there were only 18 young plants m^{-2} (on average) present across all treatments. The decline was greatest in tall standing swards where the highest initial recruitment was recorded. As earlier, UC had more seedlings surviving than CL or CR ($P<0.001$) but NS, IS or SA were not significantly different (Fig. 5-iii). HA or SR had marginally less seedlings surviving than NP ($P<0.05$) except under CR when there were more seedlings surviving in HA than SR (Fig. 6-iii). The exponential rate of decline from emergence to week 24 was higher in uncut treatments than cut treatments ($P<0.05$; $R^2 = 0.54$) but remained the same in other treatments.

There were no young plants surviving in any treatment by March 2007 (52 weeks after emergence); the remaining plants all died during their first drier than average summer.

Experiment 2 (2007-8)

In a similar manner to Experiment 1, in Experiment 2 there was recruitment of *P. aquatica* seedlings during early March 2007 (Fig. 5-i) after some rain in January (54 mm) and above average rainfall in February (90 mm) (Fig. 1). Experiment 2 did not result in as many seedlings as Experiment 1 but there were seedlings across all treatments and not only in UC, the more successful treatment in most of Experiment 1 plots. Flowering and seed set was low in Experiment 2 (Table 1) before emergence was observed. The highest seedling number recorded was 198 seedlings m^{-2} in UC x IS x HA. Recruitment of seedlings occurred in all treatments where 42% of the plots recorded between 11 and 100 seedlings m^{-2}, but nearly a third of all plots (26 out of 108) failed to record any seedlings. More seedlings were recorded in UC and CR than in CL ($P<0.001$) (Fig. 5-i). UC and CR did not significantly differ though UC had slightly more seedlings. In a similar manner to Experiment 1, recruitment of seedlings occurred more in IS and SA than in NS ($P<0.001$). No difference in seedling numbers occurred between IS and SA (same as Experiment 1) except within UC (more seedlings in IS than SA) and CL (more seedlings in SA than IS) ($P<0.001$) (Fig. 6-i). In contrast to Experiment 1, NP, HA and SR did not differ in seedling numbers except under UC when fewer seedlings germinated in HA or SR than NP ($P<0.05$). There were insignificant numbers of seedlings of species other than *P. aquatica*. Low numbers (1-5 m^{-2}) of other seedlings were observed for legumes, broadleaves and *D. glomerata*. No apparent trend across treatments was observed for these seedlings.

Experiment 2 had lower mortality rate in terms of young plant survival than Experiment 1 (Fig. 5-ii,iii,iv). Though there was far less recruitment (27 seedlings m^{-2} on average across the site), the decline through the year was only gradual. At 24 weeks after emergence (early spring), there were an average of 6 young plants m^{-2} remaining across the site. All differences across treatments during emergence continued through young plant survival at 6 weeks and 24 weeks after emergence (Fig. 5-ii,iii and Fig. 6-ii,iii). Some young plants died but there were still 3 young plants m^{-2} across the site at the end of their first summer (52 weeks after emergence). The treatment with UC x IS x HA had the highest number of young plants (20 m^{-2}) surviving. This treatment also had the highest initial recruitment. Similar numbers of young plants were surviving in UC, CL or CR (Fig. 5-iv). Within UC, fewer seedlings were present in HA or SR than NP whereas more young plants were surviving in HA within CL ($P<0.05$) (Fig. 6-iv). IS or SA had more young plants surviving than NS ($P<0.001$), a trend which had continued since the first emergence. No significant difference was observed in the exponential rate of decline from emergence to week 52 in all the treatments.

[Fig. 5]

[Fig. 6]

Discussion

This study investigated low-cost strategies for pasture rehabilitation by identifying practices that encouraged recruitment of *P. aquatica* within existing swards. The approach taken was to investigate management options that encouraged seed set, to prepare more suitable sites for seedling recruitment and to identify better post-emergence tactics that aided young plant survival in the short- to medium-term. In these field experiments there was a successful recruitment of *P. aquatica* with limited recruitment of the less-desirable species. This study was based upon previous observations (Lodge 1981; Dowling *et al.* 1996) that the better time for recruitment of perennial grasses would be in late summer, early autumn, following seed set, as previous research (Winkworth 1971; Silcock *et al.* 1990; Lodge 2004) suggested the seed bank for perennial grasses was minimal and that later in autumn the dominance of annual grasses and forbs could out-compete the weaker perennial grass seedlings. In this study measurements were taken in late summer or early autumn, late winter and through to the next summer where the only seedlings of perennial grasses found were those that had emerged late in the previous summer. The general hypotheses were therefore substantiated.

A single recruitment event of *P. aquatica* seedlings was observed in early autumn (March) in Experiments 1 and 2 after significant rainfall events in the second half of February (Fig. 1). The rainstorms in late February (57 mm in 2006; 76 mm in 2007) were the first major downfall after seed maturation and treatment application, and the seedling recruitment was the result of that event. The earlier rain in January (Fig. 1) possibly helped seed maturation and may have primed the seed for later germination (Bradford 1994; Villela 1998; Gutterman 2000). Similar observations on germination after the first major rainfall event have been reported for other grass species - *Themeda australis* (R.Br.) Stapf (Mott 1978), *Austrodanthonia* spp. (Hovenden *et al.* 2008), *Stipagrostis uniplumis* (Licht. ex Roem. & Schul.) De Winter (Zimmermann *et al.* 2008). Grass seeds germinate and emerge only in the presence of adequate soil moisture (Wilson and Briske 1979; Maze *et al.* 1993; Hamilton *et al.* 1999; Zimmermann *et al.* 2008). However, seedlings did not emerge at other times of the year even though sufficient rainfall was received for a germination event to occur, a phenomenon also reported by Zimmerman *et al*. (2008). This could be because most of the other rain occurred in the winter months (June-July) in both experiments when the mean maximum (around 10°C) and minimum (below 0°C) temperatures were at their lowest for the year (Fig. 1), temperatures considered to be less than optimal for emergence. Seedlings of species other than *P. aquatica* were present but in low numbers across all treatments. The most prominent was legumes which occurred more in uncut than cut treatments. This observation seems to be inconsistent with the findings of Leigh *et al*. (1995) concerning the effects of allelopathic chemicals from *P. aquatica* on legume establishment.

Low perennial grass seed banks were observed in this study (Table 1) even though in this case, soil cores were monitored for only two weeks after collecting them from the field. Several studies (Winkworth 1971; Mott and Andrew 1985; Silcock *et al.* 1990; Bertiller and Coronato 1994; O'Connor 1997; Lodge 2004; King *et al.* 2006) have also shown that seeds of perennial grasses were usually scarce in the soil. This is despite the fact that seed production was relatively high (>10000 seeds m^{-2}), particularly in Experiment 1 (Table 1). Lodge (2004) observed the same phenomenon of high seed production but a much lower seed bank for the perennial grasses studied. Several authors (Johns and Greenup 1976; Campbell and Gilmour 1979; Kelman *et al.* 2002) report that ant harvesting can markedly affect the availability of *P. aquatica* seeds in the soil seed bank. Seedlings did not emerge at 6, 12 or 24 weeks after the initial main emergence which supports the view that the seed bank was depleted after seed fall.

Seed set in this study was relatively high especially in Experiment 1 (Table 1) where soil moisture during flowering and seed set in summer prior to the start of the experiment was considerably wetter than conditions in Experiment 2 at the same period (Fig. 1). *P. aquatica* is known to produce large quantities of seed (Leiva and Alés 2000; Kelman and Culvenor 2007). The minimal gain in seedlings from adding seed to the pasture also suggests that the amount of seed set was able to saturate the number of microsites available for seedling recruitment. In Experiment 1 soil scarification did not increase seedling recruitment suggesting that soil already had a natural limit in the number of available microsites. In Experiment 2 soil scarifying increased seedling recruitment, but in that case it appears that overall conditions for recruitment were poorer.

The presence of established vegetation (uncut treatments) had a large positive effect on recruitment in Experiment 1. More seedlings were observed where mature plants remained uncut and the better treatments were those where *P. aquatica* plants were allowed to flower, set seed and to remain standing. Cutting tall grass and either removing the cut material or leaving it on the soil surface failed to achieve any increase in seedling numbers. There was a clear difference in seedling numbers between the treatments where swards were left untouched and where they were cut. This was an unexpected result and is in contrast to other studies (Moloney 1990; Aguilera and Lauenroth 1993; Milton and Dean 2000; Zimmermann *et al.* 2008) on perennial grasses which reported that the presence of established competitors severely suppressed seedling emergence.

Lightly scarifying the soil to create more potential microsites and reduce competition from established plants, did not lead to a significant increase in seedling emergence. This was an unexpected result that is in contrast to other studies (Kim *et al.* 1990; Hofmann and Isselstein 2004; Liu *et al.* 2008) which have shown that soil surface disturbance enhanced emergence and recruitment. One possible explanation for this difference is that the soil at the experimental site is naturally self mulching and so no benefit was gained from disturbing the soil. Other studies, such as Hofmann and Isselstein (2005), have suggested that disturbance has little effect on seedling emergence in high productivity swards. This study was conducted in a dense and productive *P. aquatica* sward. Recruitment of *P. aquatica* naturally occurs along road sides, even when fertility is low. The roadsides are ungrazed and undisturbed; it is likely that *P. aquatica* has characteristics that allow it to recruit under these conditions. Soil scarification might also apply with *Phalaris* pastures growing on different soil types and in poorer condition compared to those studied in these experiments.

Insecticide treatment did increase seedling numbers in this study (Fig. 5-i), which supports previous studies (Anslow 1958; Champ and Sillar 1961; Campbell 1966) where the use of insecticide reduced theft of seeds by ants and increased the emergence of seedlings of sown species. Though there was a slight gain at emergence from the use of insecticide (Fig 5-i), the difference between seedling numbers was overcome through the year and the numbers of surviving seedlings were similar across treatments in Experiment

2 at 52 weeks after emergence (Fig 5-iv). Therefore the benefit gained from using insecticide was fairly small. Similarly the herbicide treatment did not benefit seedling emergence when seed was not added.

In Experiment 1 no young plant survived beyond the recruitment phase supporting Lodge's (2004) results that survival of young plants is rare. However, there was a very low survival rate (1 m^{-2}) in Experiment 2, more in line with the data of Hume and Barker (1991) that some natural regeneration may normally occur for *P. aquatica*. The difficulty for survival may also be attributed to the prevailing drought. Mortality of seedlings and young *P. aquatica* plants have previously been found to be exacerbated in summers especially when drought was prolonged (Leiva and Alés 2000). Cook (1980) identified the severity and length of the dry periods as important factors affecting the growth and survival of seedlings of oversown tropical pasture species. The limited survival of young plants of other species is also reported in field populations of previous studies (Morgan 1997; Montalva *et al.* 2002; Liu *et al.* 2008). There is a reasonable chance that young *P. aquatica* plants may fail to survive dry summer conditions, highlighted by zero survival in Experiment 1 and low survival in Experiment 2. During the summer months (December to February) there were slightly better conditions in Experiment 2 (304 mm rainfall) compared to Experiment 1 (160 mm rainfall), hence few seedlings in Experiment 2 may have survived the summer months.

The general conclusion from this experiment was that minimal intervention was needed to achieve maximum recruitment and seedling survival from early autumn till early spring. The study showed *P. aquatica* recruitment was not limited by seed availability as the species normally produces high seed yields. Swards though need to be encouraged to flower and set seed which can be achieved by implementing a summer rest from grazing. Soil scarification, insecticide and herbicide use had only small effects on recruitment which is encouraging as the management during recruitment phase can remain simple and implemented at reduced costs.

Acknowledgements

The authors would like to thank Aaron Simmons and Warwick Badgery for commenting on an earlier draft of the manuscript and Helen Nicol for statistical advice.

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Fig. 1 The monthly rainfall, and maximum (Tmax) and minimum (Tmin) temperature at *Phalaris aquatica* site plotted with the 40-year average, 1968-2007 (BOM 2008).

Fig. 2 (a) Total standing herbage DM t ha^{-1}, (b) green herbage DM t ha^{-1}, (c) litter DM t ha^{-1}, (d) bare ground, and (e) plant cover percentage over time across UC (uncut), CL (cut & leave), CR (cut & remove), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for Experiment 1; predicted means from Restricted Maximum Likelihood (REML) analysis used; standard error bars presented.

Fig. 3 (a) Total standing herbage DM t ha^{-1}, (b) green herbage DM t ha^{-1}, (c) litter DM t ha^{-1}, (d) bare ground, and (e) plant cover percentage over time across UC (uncut), CL (cut & leave), CR (cut & remove), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for Experiment 2; predicted means from Restricted Maximum Likelihood (REML) analysis used; standard error bars presented.

Fig. 4 The biomass (DM t ha^{-1}) of functional groups (*Phalaris aquatica*, annual grasses = AG, C3 perennial grasses = C3 and others) across UC (uncut), CL (cut & leave), CR (cut & remove), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments in different seasons (every 3 months) for (a-c) Experiment 1, and (d-f) Experiment 2; predicted means from Restricted Maximum Likelihood (REML) analysis used.

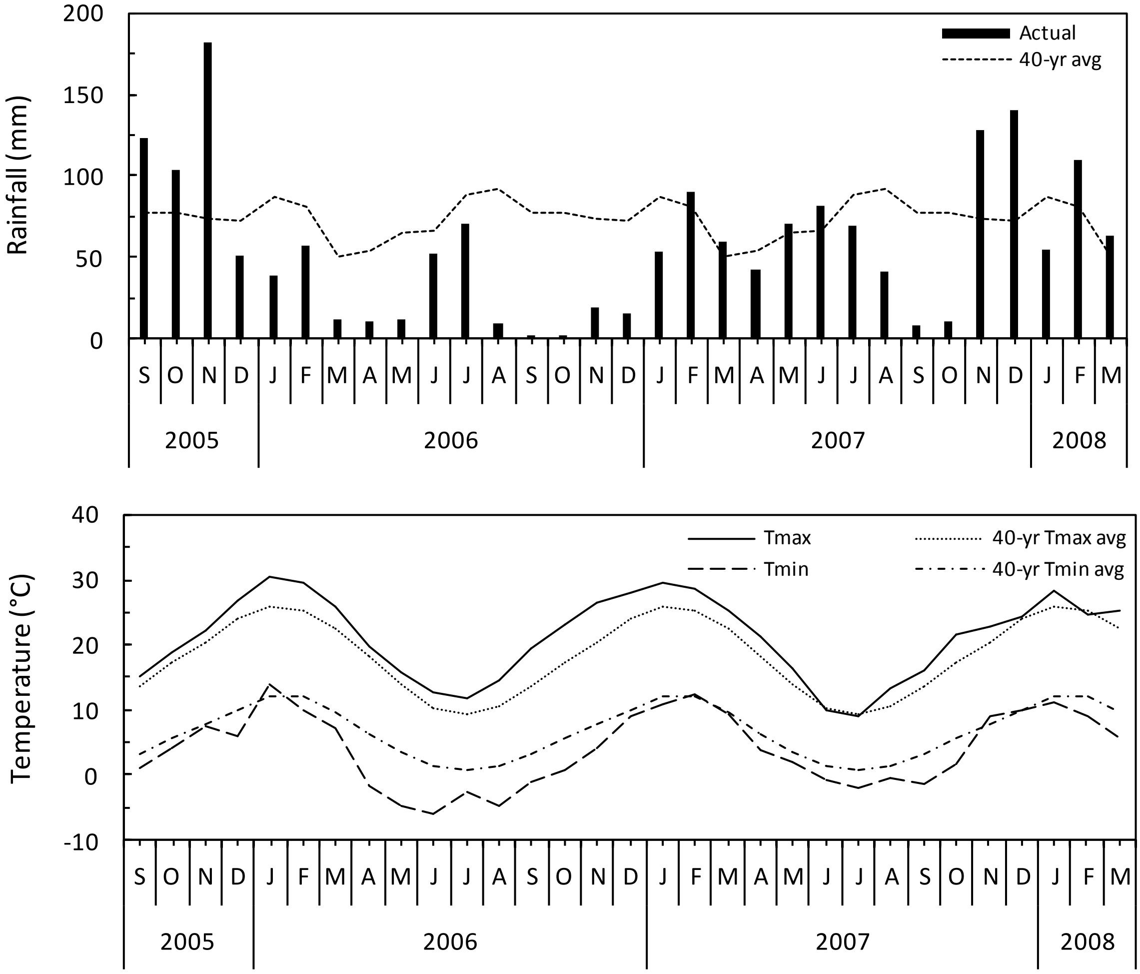
Table 1 Production of seeds at the *Phalaris aquatica* site over the 3-year experimental period (averaged over the summer months) and seedlings (m^{-2}) germinated in the glass house from the soil cores (50 mm deep) collected at the start of the experiment in January each year when seeds were maturing; temperature range used was 30/15°C; total numbers

(± standard error) in two weeks after sampling.

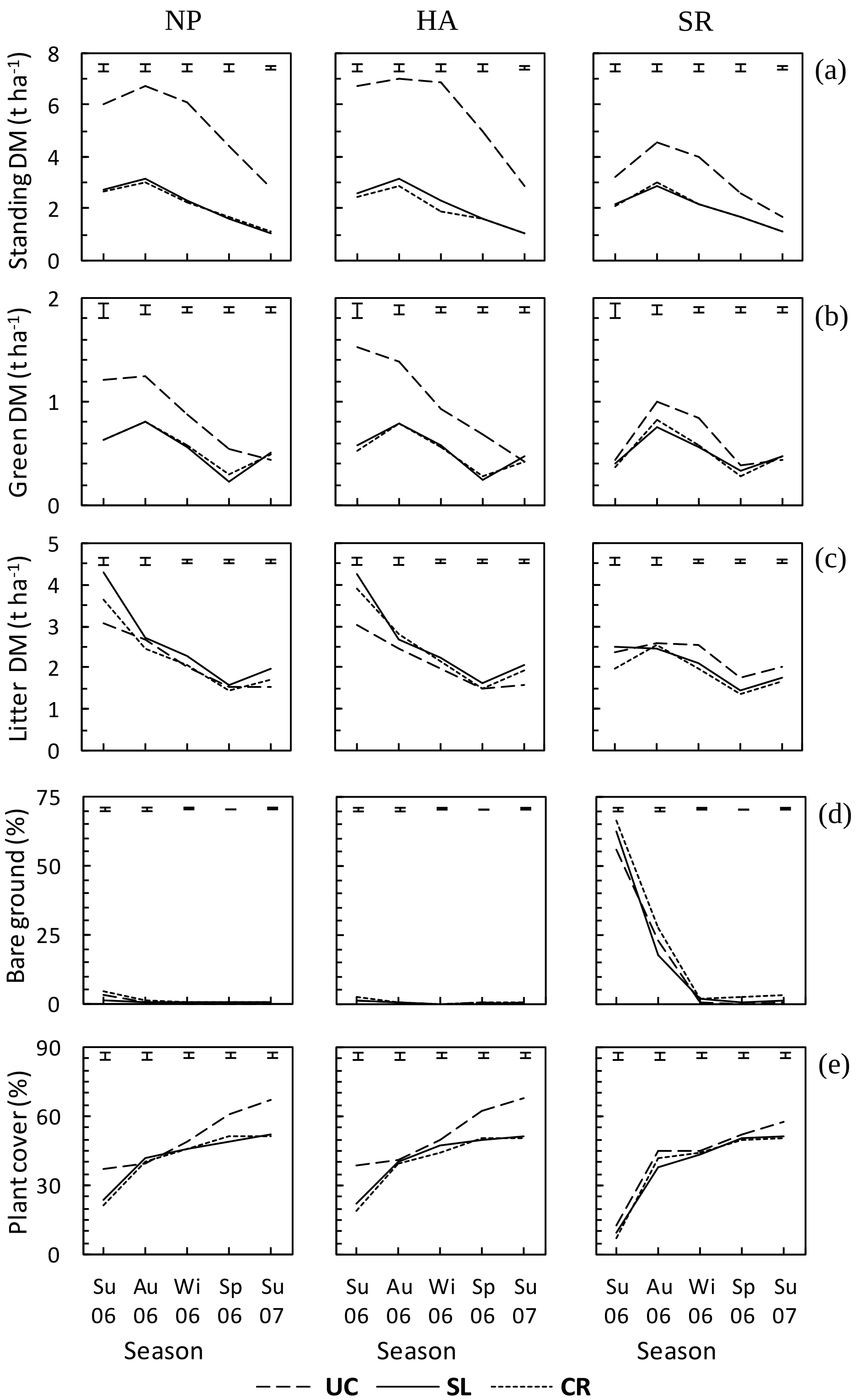
Table 2 Proportion of recruitment from natural seed set and extra seed addition across the experiment and the best recruitment with or without seed addition in any treatment and in soil scarification treatments.

Fig. 5 The average number of *Phalaris aquatica* seedlings m^{-2} (logarithmic scale, n + 1) at (i) emergence, (ii) 6 weeks after emergence, (iii) 24 weeks after emergence, and (iv) 52 weeks across UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments for Experiments 1 (2006-7) and 2 (2007-8); back-transformed means from Generalised Linear Mixed Model (GLMM) analysis used; where the same letter appears on a column within each subset of treatments in the same year, results are not significantly different, $P<0.05$; no seedlings were surviving for Experiment 1 at 52 weeks after emergence.

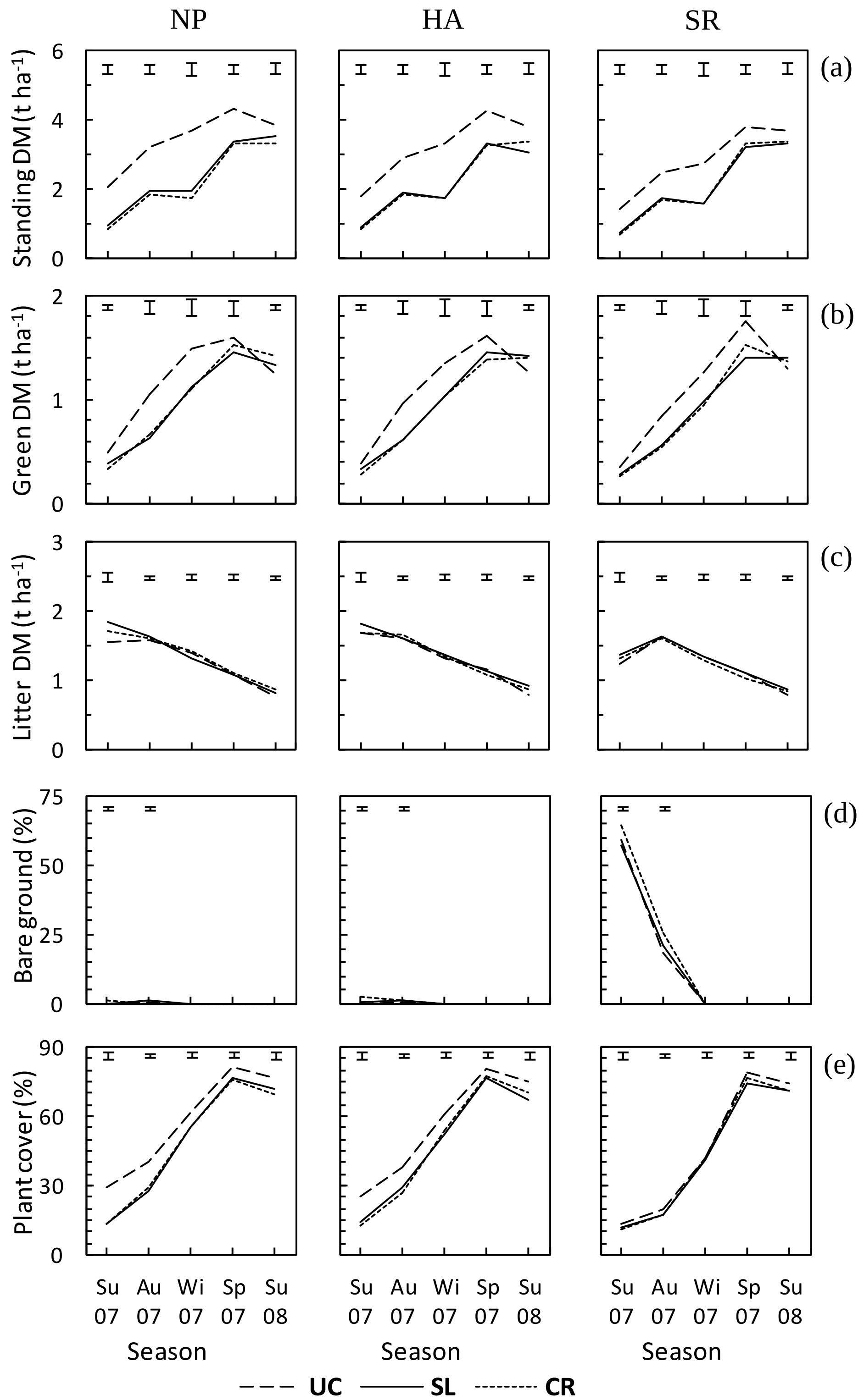
Fig. 6 The average number of *Phalaris aquatica* seedlings m^{-2} (logarithmic scale, n + 1) at (i) emergence, (ii) 6 weeks after emergence, (iii) 24 weeks after emergence and (iv) 52 weeks across all treatment combinations for UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments for Experiments 1 (2006-7) and 2 (2007-8); back-transformed means from Generalised Linear Mixed Model (GLMM) analysis used; where the same letter appears on a column within the same year, results are not significantly different, $P<0.05$; no seedlings were surviving for Experiment 1 at 52 weeks after emergence.


Rainfall (mm)
200
150
100
50
0
Actual
40-yr avg
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
2005
2006
2007
2008
Temperature (°C)
40
30
20
10
0
-10
Tmax
Tmin
40-yr Tmax avg
40-yr Tmin avg

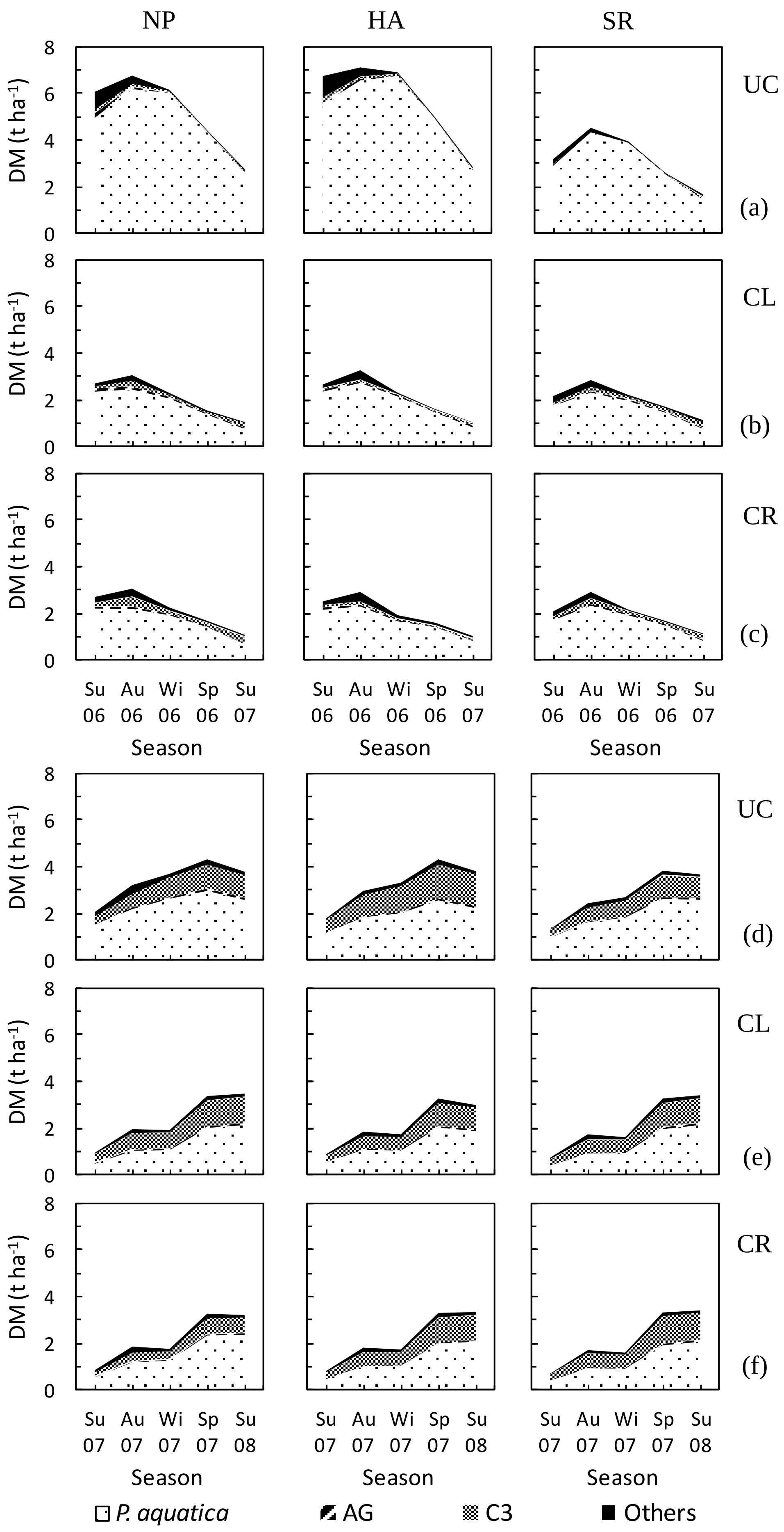
1 Fig. 1


NP
HA
SR
Standing DM (t ha-1)
Green DM (t ha-1)
Litter DM (t ha-1)
Bare ground (%)
Plant cover (%)
(a)
(b)
(c)
(d)
(e)
Su 06
Au 06
Wi 06
Sp 06
Su 07
Season
UC
SL
CR

1 Fig. 2


NP
HA
SR
Standing DM (t ha-1)
Green DM (t ha-1)
Litter DM (t ha-1)
Bare ground (%)
Plant cover (%)
(a)
(b)
(c)
(d)
(e)
Su 07
Au 07
Wi 07
Sp 07
Su 08
Season
UC
SL
CR

1 Fig. 3


NP
HA
SR
UC
CL
CR
UC
CL
CR
(a)
(b)
(c)
(d)
(e)
(f)
DM (t ha-1)
0
2
4
6
8
Su 06
Au 06
Wi 06
Sp 06
Su 07
Su 07
Au 07
Wi 07
Sp 07
Su 08
Season
P. aquatica
AG
C3
Others

1 Fig. 4

Table 1

	2006-7 (Expt 1)	2007-8 (Expt 2)
Seeds m^{-2}	12000	5400
Seed yield kg ha^{-1}	126	57
Germination (% at 25°C)	47	42
seedlings m^{-2}		
Phalaris aquatica	244 ± 101	308 ± 87
Annual grasses	138 ± 37	605 ± 117
Broadleaves	5602 ± 1147	4785 ± 830
Legumes	7958 ± 1548	5921 ± 901

Table 2

Seed			Recruitment		Best recruitment (m^{-2})	
mode	amount		m^{-2}	%	any treatment	soil scarification
Experiment 1 (2006-7)						
natural	5640 m^{-2}	59.2 kg ha^{-1}	165	2.9	1073	307
added	3986 m^{-2}	40 kg ha^{-1}	265	2.5	1032	1017
Experiment 2 (2007-8)						
natural	2268 m^{-2}	23.9 kg ha^{-1}	10	0.4	78	16
added	3986 m^{-2}	40 kg ha^{-1}	37	0.7	17	151

Note: The amounts of seed in column 3 were calculated from the data in Table 1 after accounting for germination percentages and the recruitment percentages in column 5 for the seed added treatments were calculated as the recruitment (natural - added) of the total added seed.

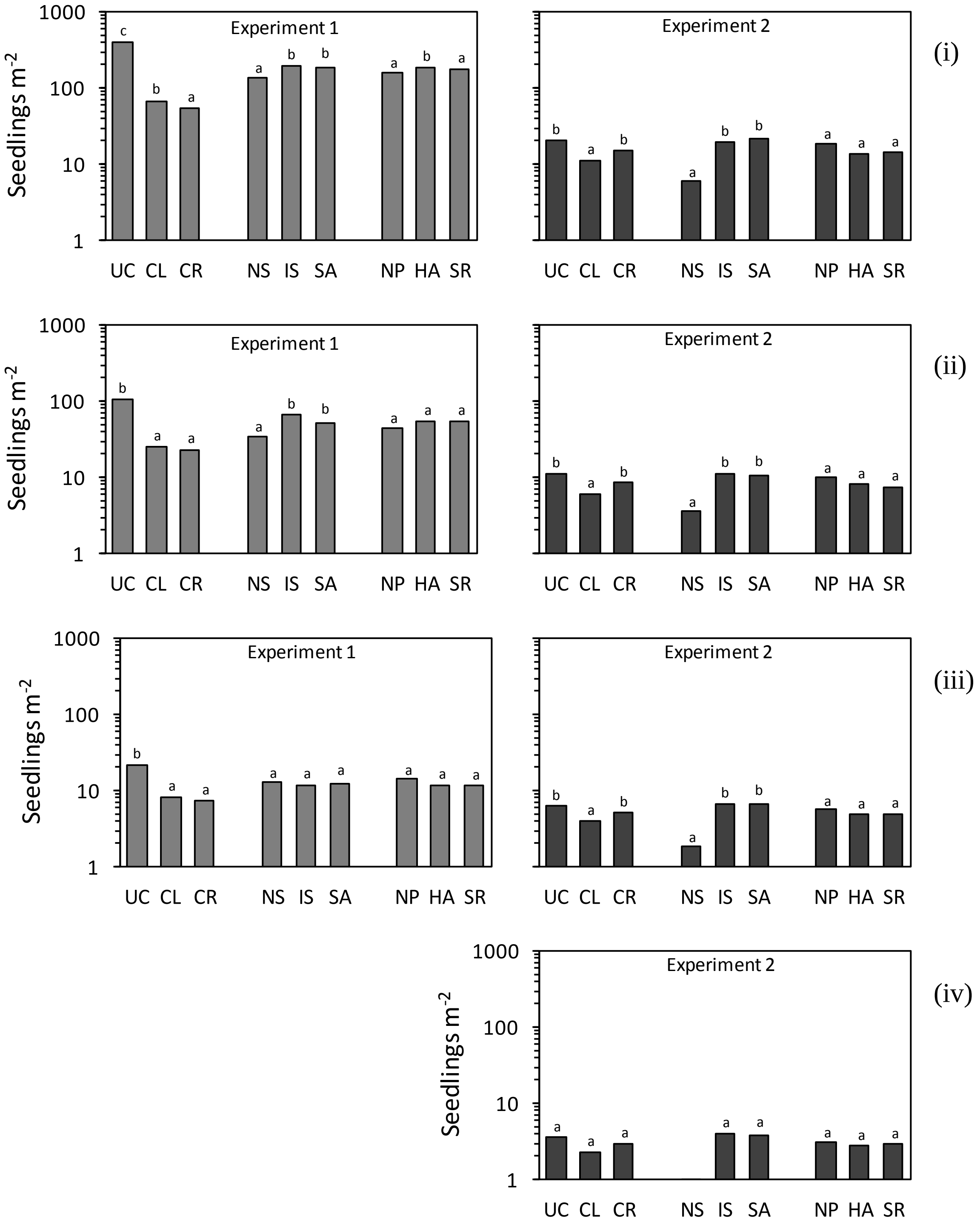

Experiment 1
Experiment 2
(i)
(ii)
(iii)
(iv)
Seedlings m-2
1000
100
10
1
UC CL CR NS IS SA NP HA SR
a
b
c

Fig. 5

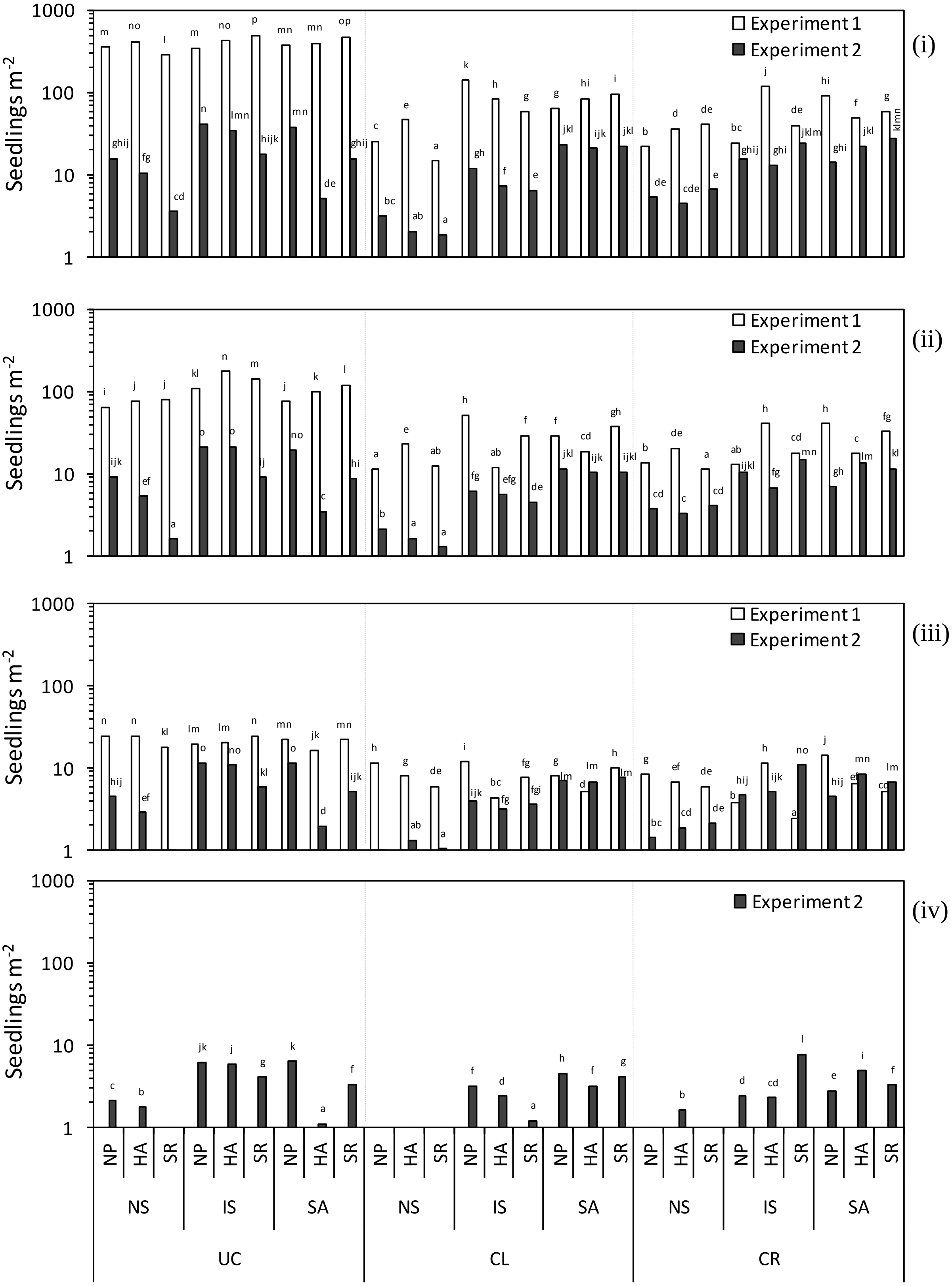

(i)
(ii)
(iii)
(iv)
Experiment 1
Experiment 2
Seedlings m-2
1000
100
10
1
NP
HA
SR
NS
IS
SA
UC
CL
CR

Fig. 6

Recruitment of *Phalaris aquatica* within existing swards 2. Effects of pasture composition

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Short title: Recruitment of *Phalaris aquatica* seedlings

Abstract. Seedling recruitment and survival are key demographic processes that determine the stability of plant populations, yet many perennial grasslands rarely achieve successful recruitment under current management practices. This paper reports on two field experiments that investigated the effects of pasture composition on the recruitment of *P. aquatica* seedlings. In both experiments, recruitment was associated with greater herbage mass (2-3 t ha^{-1}) but less green dry matter (<1.5 t ha^{-1}), more plant cover (30-70 %) and moderate litter dry matter (<2 t ha^{-1}). The limited data showed young plants at 24 weeks after emergence were surviving with moderate plant cover (30-55%), some litter dry matter (<2 t ha^{-1}) and 2-3 t ha^{-1} herbage mass of which the proportion was 80% dead. At 52 weeks emergence when the success of survival was determined, treatment effects were not discernible but the number of young plants that survived seemed to have been influenced by the presence of competitive biomass of existing plants. Experiment 1 had nil survival at 52 weeks after emergence.

Additional keywords: seedling recruitment, seedling survival, herbage mass, green dry matter, litter dry matter, plant cover

Introduction

Phalaris aquatica L., formerly known as *P. commutata*, *P. bulbosa*, *P. stenoptera* and *P. tuberosa*, is a temperate C3 perennial grass that has been widely sown in the Australian environment as a permanent pasture species since its introduction in the late 19th century (Watson *et al.* 2000). The species is recognised as the most persistent and productive grass commercially available in Australia. The grass is drought tolerant, frost resistant, competitive and robust against many serious weeds of pastures, can withstand extended periods of heavy grazing, and provides good quality forage for all types of grazing livestock (Watson *et al.* 2000). It performs well in poorly drained waterlogged soils and grows successfully on a wide range of soils but is not very tolerant of high aluminium, acidity and low fertility soils (Lamp *et al.* 2001). *P. aquatica* grows best during autumn and spring at temperatures between 15 and 25°C. There are a number of cultivars available that have now been bred for Australian conditions of low soil pH.

P. aquatica produces large quantities of seed (Leiva and Alés 2000; Kelman and Culvenor 2007), however little recruitment within *P. aquatica* stands has been reported (Dowling *et al.* 1996; Virgona and Bowcher 2000; Lodge 2004). One of the major factors is *P. aquatica* seedlings are susceptible to competition from annual grasses (Virgona and Bowcher 1998; Lodge 2000) and sown legumes (McWilliam *et al.* 1970; Lamp *et al.* 2001) during the establishment phase. Heavy loss of seeds through ant predation contributes highly to low recruitment in *P. aquatica* swards (Campbell 1966; Campbell and Gilmour 1979; Kelman *et al.* 2002). The adult *P. aquatica* plants are able to colonise new spaces that further limits recruitment opportunities in exiting pastures (Campbell *et al.* 1981; Lodge 2004). In contrast, *P. aquatica* spreads readily by seedling recruitment along ungrazed roadsides in south-eastern Australia (Kelman *et al.* 2002; Lodge 2004). Recruitment obviously occurs at times under natural conditions, but the mechanisms are poorly understood.

P. aquatica has a weak seedling that increases the cost of sowing pastures, as more effort needs to be done to minimise competition. These less vigorous seedlings limit the ability of low density *Phalaris* stands to increase their density. Management practices to increase sward densities have not been developed. Cultivars have been selected with more vigorous seedlings but they are still not as competitive as many annual grasses (Lodge 2000). More recently developed cultivars are often less persistent (Lodge 2000; Lodge and Orchard 2000) than the original introduction, which increases the need to determine management practices that can improve sward densities. Similar problems exist with *Festuca arundinacea* Schreb., another commonly sown and productive introduced perennial grass (Lazenby 1997).

The aim of experiments reported in this paper was to better understand the mechanisms of *P. aquatica* recruitment and investigate the effects of pasture composition on recruitment and survival of seedlings within existing swards.

Methods

Site

The experiment was located at Orange on the Central Tablelands of New South Wales (149°07' E, 33°14' S) in the same pasture described by Thapa *et al.* (2010) in which a complete site description is provided. Briefly, the site had an elevation of 826 m (a.s.l.), an average annual rainfall of 890 mm and a Vertosol soil which was a very dark grey colour with a light clay surface texture. The experiment was established in October 2005 and continued until March 2008.

Experimental design

The experimental design was based upon a factorial combination of 3 seed delivery mechanism treatments x 3 seed treatments x 3 site preparation treatments as described in Thapa *et al*. (2010). The experiment was repeated over two years (Experiments 1 and 2) on separate plots to capture the establishment in two different years; Experiment 2 (second year) being a repeat of Experiment 1 (first year) on an adjoining site.

Treatments

Treatments were designed within a range of practices to investigate the recruitment process and were within the reasonable limits of what could be implemented on farms. They were designed to generate variations in the amount of plant competition, cover / shading, litter, bare ground and surface soil moisture levels, all factors which are known to influence seedling recruitment *in situ* (Dowling *et al.* 1971; Wilson and Briske 1979; Fowler 1986; Moloney 1990; Lauenroth *et al.* 1994; Milton and Dean 2000; Lodge 2004; Zimmermann *et al.* 2008). Treatments were applied at the start of summer in January 2006 for Experiment 1 and in January 2007 for Experiment 2.

The 3 seed delivery mechanism treatments were: uncut or control (UC), cut and leave (CL), and cut and remove (CR). The seed treatments were: no seed addition or control (NS), insecticide application (IS) and seed addition (SA). The 3 site preparation treatments included: no preparation or control (NP), herbicide application (HP) and scarify and rake (SR). For a complete description on treatments refer Thapa *et al*. (2010).

Plot layout

The same plot layout was used as was outlined in Thapa *et al*. (2010) and a brief summary is presented here. All plots were 2 x 2 m within which 0.9 x 0.9 m area in the centre was permanently marked for routine measurements. The 0.9 x 0.9 m area was subdivided into nine 0.3 x 0.3 m contiguous quadrats (arranged in a 3 x 3 square) and used for biomass and plant species composition measurements. Each quadrat was further divided into 81

0.1 x 0.1 m sub-quadrats arranged within the 0.9 x 0.9 m permanently marked area. Seedling numbers were recorded in these sub-quadrats. Treatments were applied over the whole 2 x 2 m plot.

Measurements

The same measurements were used as was described in Thapa *et al*. (2010) and a brief summary is presented here. Dry weight ranks of the 3 most abundant species and the total dry matter (DM) of all species were estimated using BOTANAL procedures (Tothill *et al.* 1992). Dry weights of standing DM (t ha^{-1}) and litter DM (t ha^{-1}) were estimated separately. Pasture biomass was sampled every 3 months in the late summer (February), autumn (May), winter (August) and spring (November) of each experimental year. Plant cover, litter cover, and bare ground percentages were visually estimated using ratings in 5% increments (*e.g.* 0, 5, 10, to 100). Initial seedling counts were recorded within 2-3 weeks of seeds germinating (after treatment application) and then after approximately 6, 24, and 52 weeks.

Analyses

A General Linear Multiple Regression using a quadratic model (GLM) was used to identify associations of seedling recruitment and young plant survival with functional plant groups DM (t ha^{-1}), green DM (t ha^{-1}), litter DM (t ha^{-1}), bare ground (%) and plant cover (%). Sequential Bonferroni correction was used for alpha (α) level adjustment to account for multiple comparisons. Cross-correlations in regressions were investigated to avoid the confounding of factors. Regression tree analysis using least squares was then used to investigate the relative importance of these factors on recruitment and young plant survival and identify the order in which factors were more important. No more than 10 splits were permitted and the terminal nodes had to contain 5 or more units. Minimum split and split proportions were set at 0.05.

There were no young plants surviving at the end of Experiment 1 in early autumn 2007 due to the drought. Survival analysis for Experiment 1 was based on the data collected before the summer in August 2006 (24 weeks after first emergence). Seedling data were square root transformed ($\sqrt{}$ (seedling m^{-2} + 0.5) for GLM analysis. All statistical analyses were done using GenStat 9.1® (Payne *et al.* 2006) except the regression tree analyses that were done using Systat 12® (Systat Software Inc. 2007).

Results

Biophysical factors affecting recruitment

The imposed treatments altered the level of plant cover, bare ground, litter, biomass, green and functional group biomass. Treatment effects were significant (Thapa *et al.* 2010) hence the seedling numbers that resulted were likely to be a reflection of the available microsites or resource space and competitive environment created in each treatment. These relationships are examined in this and the next sections in a series of figures, tables and regression (decision) trees. Data are only shown over the range in values of each data subset (per treatment) where a significant relationship was found. Within each significant relationship for the factor of interest, zero values were dropped from the figures for clarity. Finally regression trees were used to determine the relative importance of significant factors. Results are presented at two levels: (i) to investigate physical microsite descriptors (*i.e.* plant cover, bare ground, litter) for their influence on recruitment and (ii) to investigate effects of competition (*i.e.* green DM, plant functional groups). Within that framework each significant factor is described to identify the limits or quantify the factors where better results in seedling numbers were obtained. Because uncut and cut (CL and CR) treatments appeared to behave differently, results are presented accordingly.

Bare ground (%) is generally related to plant cover (%) but a high correlation was not found for the data (*not presented*) although more plant cover (%) was associated with less bare ground (%). Therefore both bare ground (%) and plant cover (%) were considered in these analyses. A significant ($P<0.001$; $R^2 = 0.64$) positive relationship was found between litter cover (%) and litter DM t ha^{-1}. Because litter DM, compared to litter cover (%), featured in more significant relationships in the initial analysis, litter DM was considered the more important factor and was used in the analyses presented here. Litter DM was more of an accurate measure due to corrections from calibration cuts whereas litter cover (%) were only visual estimates. The combined area of litter cover (%) and bare ground (%) represents the space available for a seedling to emerge. As there was some overlap between litter cover (%) and plant cover (%) by definition, another variable (residual plant cover %) was generated by subtracting the sum of litter cover (%) and bare ground (%) from 100%. Residual plant cover (%) would then be an estimate of the area where the chances of a seedling emerging would be small. But a highly significant ($P<0.001$; $R^2 = 0.81$) positive correlation was observed between plant cover (%) and residual plant cover (%) hence only the analyses reverted to the direct estimate of plant cover (%). This also suggests that the overlap between plant and litter cover (both as %) was small and not of great concern in biasing analyses.

Experiment 1 (2006-7)

Seedling numbers were maximised (~1300 m^{-2}) when plant cover was roughly 45% or greater (Fig. 1-1,3 – UC x NP with NS or IS) or had lower maxima (~250 m^{-2}) at around 30% plant cover in cut treatments (Fig. 1-9,13 - CL x SA x HA or CR x IS x NP). Collectively this suggests that overall plant cover needs to be ~30-70% to maximise initial seedling numbers. These analyses suggest that the protection provided by plant cover was more important than bare ground for recruitment.

There was a declining trend in initial seedling numbers with the increase in litter DM but seedling numbers were at the maximum (~80 m^{-2}) when litter DM was between 2-3 t ha^{-1} (Fig. 1-6,14,15 - cut treatments that had NS x SR, IS x HA or SA x NP). These significant relationships were only observed in cut treatments indicating the importance of litter in providing physical sites for recruitment when there was less plant cover.

Green DM around 1 t ha^{-1} maximised initial seedling numbers (~600 m^{-2}) in UC x NS x HA (Fig. 1-2). Within cut treatments seedling numbers were maximised (~200 m^{-2}) when green DM was <0.5 t ha^{-1} (Fig. 1-7,8,16). Seedling numbers declined with increasing green DM except in CR x SA x HA (Fig. 1-16) where there was a slight upward trend but the overall effect of green DM was negative within both cut and uncut treatments. This suggests that higher green DM competition (>1-1.5 t ha^{-1}) is unfavourable to emerging seedling numbers.

Maximum seedling numbers (~700 m^{-2}) were observed when *P. aquatica* total DM was around 4 t ha^{-1} or more in the UC x SA x NP (Fig. 1-4). Lower seedling numbers (~100 m^{-2}) were associated with 2-3 t DM ha^{-1} (Fig. 1-11,12,17 - cut treatments that included NS x NP, NS x SR or SA x SR). Seedling numbers were also maximised when small amounts (0.3-0.5 t ha^{-1}) of functional groups other than *P. aquatica* were present, particularly annual grass DM (Fig. 1-5 – UC x SA x SR) and others DM (Fig. 1-10 – CL x SA x SR). Collectively this suggests that seedling numbers would be maximised if overall standing DM was above 2-3 t ha^{-1}. This indicates the presence of existing vegetation was a beneficial factor for seedling emergence.

In general, the examination of individual relationships indicates that greater herbage mass (>2-3 t ha^{-1}) but less green DM (<1.5 t ha^{-1}) and more plant cover (30-70%) or litter DM (~2-3 t ha^{-1} or 40% as litter cover) were associated with maximum numbers of *P. aquatica* seedlings. Use of regression trees across all treatments showed that when all factors were included, the total standing DM and plant cover were the more important factors determining overall seedling numbers (Fig. 2-a). Where DM was less than 3.9 t ha^{-1} seedling numbers were approximately 20% (~100 m^{-2}; n = 709) of those where the DM was >3.9 t ha^{-1}. Plant cover was the next most important criterion in determining seedling numbers. Seedling numbers were doubled (~800 m^{-2}; n = 77) when plant cover was above 45% compared with below 45%. Green DM and litter did not emerge as major factors in the regression tree analyses, suggesting that they may have been significant in the analyses of individual, but dropped out of this stepwise regression analysis. There was an increasing relationship between seedling numbers and total DM but a decreasing relationship between seedling numbers and green DM. It is more likely that the adverse effects of high green DM was so strongly linked with the UC treatment and so poorly linked with the CR treatment that it dropped out of the general pooled model. The proportional reduction in error was reasonable (0.39) indicating that there were still a range of additional factors that determined seedling emergence, but they were probably not consistent across all treatments.

[Table 1]

[Fig. 1]

[Fig. 2]

Experiment 2 (2007-8)

Seedling numbers (~120 m^{-2}) were greatest when plant cover was around 40% within UC x SA x NP (Fig. 3-4). In cut treatments, maximum seedling numbers (~60 m^{-2}) were obtained when plant cover was 15% (Fig. 3-9,10,17 - treatments with IS x SR or SA x NP / HA). Collectively this suggests that overall plant cover needed to be 15% or greater; this range was lower than Experiment 1 but herbage mass was less in Experiment 2 (Thapa *et al.*

2010). However, similar to Experiment 1 maximum seedling numbers were associated with higher plant cover within a subset of treatments. Consistent with Experiment 1, bare ground was less significant than plant cover as a source of sites for recruitment in these analyses except when bare ground (10-15%) maximised seedling numbers (~50 m^{-2}) in CR x IS x HA (Fig. 3-14).

Seedling numbers were maximised (~70 m^{-2}) when litter DM was around 1.5 t ha^{-1} (Fig. 3-2,3 - UC x IS with HA or SR; Fig. 3-11,16 - CR with NS x NP or SA x SR). In CL, seedling numbers were maximum (~40 m^{-2}) when litter DM was ~2 t ha^{-1} (Fig. 3-8).

There was lack of relationship between seedling numbers and green DM in Experiment 2 compared to Experiment 1. This is not really surprising as there was very little variation in green DM in the summer and autumn of 2007 (start of Experiment 2) compared with the summer and autumn of 2006 (start of Experiment 1) (Thapa *et al.* 2010). However, when green DM was 0.4-0.6 t ha^{-1} seedling numbers were maximised (~50 m^{-2}) in CL x SA x NP (Fig. 3-10); this was similar to Experiment 1 when ~0.4 t ha^{-1} green DM was favourable within the same treatment (Fig. 1-8). This suggests that presence of some green DM (~0.5 t ha^{-1}) could be beneficial to seedling emergence but higher may be unfavourable.

When *P. aquatica* DM was around 3 t ha^{-1}, maximum seedling numbers (~70 m^{-2}) were observed (Fig. 3-1 - UC x NS x HA). Within cut treatments seedling numbers were maximised (100 m^{-2}) at ~1 t ha^{-1} *P. aquatica* DM (Fig. 3-5,6,7,12,13,15). This suggests existing vegetation was a positive influence on seedling emergence as it was in Experiment 1. The bulk of standing DM was dead (70%) hence the presence of existing vegetation could be more important as protection for initial seedlings.

In general, there were lower seedling numbers in Experiment 2 than Experiment 1.

However, variation in seedling numbers was associated with similar factors. Plant cover (>15%), herbage mass (1-3 t ha^{-1}), and less green DM (~0.5 t ha^{-1}) or litter DM (~1.5 t ha^{-1}) maximised *P. aquatica* seedling numbers as shown in the individual significant relationships. Use of regression trees across all treatments and factors found the only important factor in predicting seedling numbers at emergence that applied across the whole experiment was total standing DM (Fig. 2-c). The cut off value was 2.7 t ha^{-1} below which 24 seedlings m^{-2} (n = 911) and above which 81 seedlings m^{-2} (n = 61) were present on average. The lower values than for Experiment 1 reflected the drier conditions during the summer prior to the start of experiment 2, which resulted in higher number of seed set.

[Table 2]

[Fig. 3]

Biophysical factors affecting young plant survival

24 weeks after emergence

Experiment 1 (2006-7)

The greatest number of young plants were surviving where plant cover was around 55% (~60 m^{-2}; Fig. 4-4 – UC x SA x SR) or at around 30% plant cover (~20 m^{-2}; Fig. 4-13 – CR x IS x SR). Though treatments were different, the range in plant cover fell within where maximum seedling numbers initially emerged indicating plant cover may have to be around 30% or 55% for continued survival of young plants.

Surviving young plant numbers were at the maximum (~50 m^{-2}) when litter DM was around 1.7 t ha^{-1} (Fig. 4-1 - UC x NS x HA) or around 3 t ha^{-1} in the seed addition treatment (Fig. 4-3 - UC x SA x HA). In the latter case the number of young plants surviving was ~80 m^{-2}. These differences suggest there may be an important interaction applying the amount of litter associated with maximum seedling survival increases with greater initial seedling numbers at emergence. These litter DM values were similar to those that optimised initial seedling emergence but in this case significant relationships were only found in uncut treatments whereas significant relationships were found in cut treatments at emergence.

Within UC x SA x NP where green DM was ~1 t ha^{-1}, maximum numbers (~90 m^{-2}) of young plants were surviving (Fig. 4-2). When ~0.7 t ha^{-1} green DM was present, maximum surviving numbers (~40 m^{-2}) were lower (Fig. 4-8,10,12 - cut treatments with SA x NP or NS / IS x HA). Green DM therefore needs to be ~0.7-1 t ha^{-1} to maximise young plant survival. This level of green DM also maximised initial seedling numbers (albeit in different treatments) which indicates lower green DM may be preferable to encourage seedling emergence and young plant survival in the initial stages.

The significant relationship for *P. aquatica* DM was observed only in cut treatments where ~3 t ha^{-1} had maximum young plants (~50 m^{-2}) surviving (Fig. 4-5,7,9,11). In addition surviving young plants were at maximum when 0.4 t ha^{-1} of annual grasses DM (Fig. 4-4 - UC x SA x SR) and 0.2 t ha^{-1} of others functional group DM (Fig. 4-6 - CL x NS x SR) were present. Collectively this suggests retaining standing DM of 3 t ha^{-1} (of which 80% was dead) would be beneficial to young plant survival.

In general, existing standing DM (~3 t ha^{-1}) with lesser green DM (<1 t ha^{-1}), plant cover (30-55%) or litter DM (~2-3 t ha^{-1}) maximised survival of young *P. aquatica* plants; these values were more or less similar to where better results for initial seedling recruitment were obtained. However across all treatments regression tree analysis indicated that green DM was associated with overall number of young plants surviving 24 weeks after initial emergence (Fig. 2-b). Where green DM was <0.7 t ha^{-1}, average young plants surviving were 9 m^{-2} (n = 606) and above 0.7 t ha^{-1} green DM had on average 31 m^{-2} (n = 366) young plants surviving.

[Table 3]

[Fig. 4]

Experiment 2 (2007-8)

Analyses of individual factors found that the greatest number of young plants (~20 m^{-2}) survived in uncut treatments when the plant cover was ~55% (Fig. 5-3 - UC x NS x SR). In UC x HA, plant cover of 70-80% resulted in highest plant numbers, though the actual numbers were low (10 m^{-2}) (Fig. 5-2). This range in plant cover was slightly higher than Experiment 1 and that which maximised the initial emergence in Experiment 2 but herbage mass then declined in Experiment 1 whereas the reverse applied in Experiment 2 (Thapa *et al.* 2010).

Litter DM around 1.7 t ha^{-1} maximised the number of surviving young plants (~20 m^{-2}) (Fig. 5-8 - CR x IS x HA); this was similar to Experiment 1 and in Experiment 2 where maximum seedlings initially emerged but occurred in different treatments. This suggests that maintaining a litter layer (~2 t ha^{-1}) would provide microsites needed for seedling emergence and through initial stages of young plant survival.

The highest number of young plants occurred where *P. aquatica* DM was ~5 t ha^{-1} (~15 m^{-2}; Fig. 5-1 - UC x NS x NP) or at around 2 t ha^{-1} within CL x IS x NP (~25 m^{-2}; Fig. 5-6). Surviving young plants were at a maximum (~20 m^{-2}) when C3 functional group DM (other than *P. aquatica*) was ~2.3 t ha^{-1} (Fig. 5-4 - UC x SA x SR) or when ~0.2 t ha^{-1} of others functional group DM was present in cut treatments (Fig. 5-5,7 - CL with NS x SR or IS x NP). Collectively this suggests surviving seedling numbers would be maximised when ~2 t ha^{-1} of standing DM was present, which was lower than Experiment 1 but there was higher green DM across all treatments in Experiment 2 (~50% compared to 20% in Experiment 1).

In general, lower numbers of young plants were surviving Experiment 2 than were observed in Experiment 1 but surviving young plants were associated with similar factors. Plant cover (~55%), litter DM (~2 t ha^{-1}) or standing DM (~2 t ha^{-1}) maximised survival of young plants 24 weeks after emergence. Regression tree analysis however failed to identify any one most important factor; this may be because of the low survival rate for these young plants.

52 weeks after emergence

Experiment 1 (2006-7)

No young plants survived to this stage.

Experiment 2 (2007-8)

Numbers of surviving young plants were greatest (~10 m^{-2}) when plant cover was between 60-65% (Fig. 5-11 - CR x IS x HA), *P. aquatica* DM between 3.1-3.8 t ha^{-1} (Fig. 5-12 - CR x SA x HA), C3 functional group DM ~3.6-4.0 t ha^{-1} (Fig. 5-9 - UC x IS x NP) or others functional group DM around 0.3-0.4 t ha^{-1} (Fig. 5-10 - UC x SA x SR). Though significant relationships were not observed, other treatments had similar numbers of young plants still alive (Thapa *et al.* 2010), which indicates that factors other than microsites (defined by plant cover, litter, bare) and competition (green, functional groups) may be affecting survival at 52 weeks after emergence.

[Table 4]

[Fig. 5]

Discussion

This study was done to better understand the recruitment process of *P. aquatica* within existing pastures by investigating the effects of pasture composition on recruitment and survival of seedlings. The approach taken was to apply treatments which created variation in the amount of plant competition, cover, litter, bare ground and surface soil moisture levels, factors that influence seedling recruitment. In these field experiments, it was clear that seedling recruitment was dependant on seed availability with other factors (herbage mass, bare ground, little layer, soil moisture) playing important roles and just prior to an opportunity for recruitment it required a suitable rainfall event soon after mature seed fall and sward conditions that help maintain a suitable microclimate around germinating seeds.

Herbage mass was found to be a positive influence on seedling densities throughout Experiment 1. The ratio of dead to green vegetation was approximately 4 to 1 across all treatments when seedlings initially emerged (Thapa *et al.* 2010). The standing DM may then be positively influencing seedlings by providing cover and protection rather than creating competition - even though competition is still a reality as too much green biomass depressed seedling numbers. During the initial stage of recruitment, tall uncut swards probably provided higher moisture retention due to shading (acting as nurse plants), a greater boundary layer resistance to air movement and slightly lower vapour pressure deficits, which helped the seedlings to germinate in comparison to open (cut) swards or those where the total biomass was the same but had been cut and put as litter on the soil surface. This effect may arise from subtle changes in humidity at ground level; changes that are very difficult to measure. This was in contrast to CL, where the plant material was slashed and laid on the soil surface which could have been expected to reduce evaporation and enhance germination, but evidently did not. In 2006, standing swards with >3 t DM ha^{-1} resulted in higher numbers of seedlings establishing than those that were cut with the same biomass lying on the ground. This suggests that the microclimate at microsites where seedlings emerge may be initially more important than competition for soil moisture and nutrients, for this species under dry conditions. This observation is supported by Scott (1997), which shows that the constraints during germination and emergence are different to the constraints during the seedling growth and survival phase brought about by competition for soil moisture, nutrients and light.

Experiment 2 did not show significant differences in seedling numbers between the cut and uncut sward, in contrast to Experiment 1. This could reflect the difference in starting herbage mass which was much lower in Experiment 2 (1.1 t ha^{-1} compared to 3.4 t ha^{-1} in Experiment 1 - Thapa *et al*. 2010) with the result that cutting or not cutting may not have created sufficiently large differences between the swards in whatever factors affected seedling recruitment. Leaving the sward intact in Experiment 2 did not reduce seedling establishment, hence there appears to be no justification for slashing paddocks after seed set to enhance recruitment. In earlier studies of semiarid grasslands (O'Connor 1996; Bisigato and Bertiller 2004) retaining the existing vegetation had little or no negative effects on early stages of seedling recruitment. Few studies (Vilà and Lloret 2000; Snyman 2004) have reported perennial grass seedlings to even survive and perform better when located close to established vegetation.

The presence of larger quantities of litter (>2-3 t ha^{-1}) resulted in fewer seedlings in this study. This shows cutting the grass and distributing it on the surface does not help recruitment. It was anticipated that the presence of litter would maintain higher soil moisture levels and reduce the rate of drying of the soil surface (Evans and Young 1970; McWilliam and Dowling 1970; Mott *et al.* 1976) thereby enhancing germination (Fowler 1986). Lodge (2004) reported a positive relationship between the presence of litter and seedling emergence of *P. aquatica* cv. Sirosa, though the effect was different at various times of the year and with different soil type. That study (Lodge 2004) included two soil types, Red Chromosol and Grey Vertosol, the latter being similar to the soil type in the present study. For the Grey Vertosol, emergence was higher with litter cover (~1 t ha^{-1})

compared with no litter and for the Red Chromosol emergence was higher in areas with litter cover in October and November but lower in July and September compared with areas with no litter (Lodge, 2004). Litter levels in this study were high compared to grazed pastures and to Lodge's (2004) study. Litter can act as a physical barrier (Facelli and Pickett 1991) preventing moisture from reaching the soil surface. There are obviously some subtleties in how best to prolong periods of favourable moisture conditions for seedling establishment. From an application perspective though, the results all support the view that simply leaving swards intact is the better solution.

The results show that legumes were minor vegetative components of these pastures which perhaps suggest that the very dry seasons and/or low fertility may have been constraining the growth of legumes. However, the presence of legumes in these pastures and their potential effects in supporting livestock production and in nourishing the companion grasses by supplying a much needed nitrogen input if fertility constraints were alleviated could not be ignored. Given reasonable seasons and sufficient soil phosphorus and sulphur, legumes can be major contributors to pasture production and quality into the future. Annual grasses were not prominent in this study but when present had a negative impact on both seedling emergence and survival rates of young plants. Annual grasses and legumes are known to compete with weak *P. aquatica* seedlings in the early stages of recruitment (Lamp *et al.* 2001). Due to their Mediterranean origins, the peak period of germination for most annual grasses is later in autumn under mild temperature conditions than applied in this study with *P. aquatica*. There is probably a narrow period between seed maturation of perennial grasses and the occurrence of a suitable rainfall event in late summer, before subsequent rainfall events and the onset of milder temperatures create opportunities for annual grasses to readily germinate and offer substantial competition to establishing perennial grasses.

While the initial establishment of seedlings may not have been hampered by competition from mature plants of any species for soil moisture, nutrients and light, as the season progressed, such competition did become more apparent. The decline in the number of seedlings (in proportional and absolute terms) within tall uncut swards was considerable but it was not the case in the cut treatments. The exponential rate of decline in seedling numbers was significant in uncut treatments (Adj $R^2 = 0.54$; $P<0.05$). The seedling density was highest in the tall uncut swards but 24 weeks after emergence, it had decreased and more or less equalled the average number in other treatments. In this study it is evident that the rate of seedling loss did vary between treatments. This indicated that no matter what the density of the seedlings, some always survive and while the rate of loss could be density dependent, there may be a threshold number that survives which does not depend upon the original seedling density.

The level of plant competition experienced by the young plants is probably best shown by the relationship with the amount of green DM. Surviving young plants (24 weeks after emergence) were present when green DM was ~1 t ha^{-1} for Experiment 1. The possible explanation could be that the existing adult *P. aquatica* plants accounted for most of green DM which would have competed more with the young plants than the newly establishing annual plants. At this same time, the existing herbage mass was positively related to young plant survival. It is likely that there was an association between the conditions allowing green growth and those same conditions allowing the survival of young seedlings. Also, at 24 weeks after emergence, young plant survival was positively correlated with moderate plant cover (30-55%), some litter (<2 t ha^{-1}) and 2-3 t ha^{-1} herbage mass of which 80% was classified as dead. These conditions may have posed minimal competition to surviving young plants and thereby reflect a modification to the sward structure that helped shelter seedlings through autumn and winter. At 52 weeks after emergence, surviving young plants, though present in a very low number, were still associated with standing DM (~3.5 t ha^{-1}) but the competitive factor represented by green DM was around 1.3 t ha^{-1}. Cook and Ratcliff (1985) have even shown reduction in the early growth of the sown species by a factor of 45 when competition was imposed from both shoots and roots together. During dry summers, Badgery *et al*. (2008) found that seedlings of *Nassella trichotoma* (a perennial grass weed) failed to survive even at low levels of perennial grass (0.5 t DM ha^{-1}) competition.

The general conclusion from this study was that retaining vegetation to provide cover and protection rather than competition for emerging seedlings generated the better results for recruitment. High levels of litter on the ground was ineffective in getting more seedlings which showed vertical litter (existing vegetation) was preferable to horizontal litter (laying on the ground) in encouraging recruitment for this species under dry conditions. Doing nothing other than imposing a complete rest during summer may be the best option for managing *Phalaris* pastures as intact swards produced the better results whereas the loss of recruited seedlings was greater in uncut swards. It may well be that the already existing 40 plants m^{-2} of mature *P. aquatica* plants were likely to have prevented the survival of these seedlings much beyond spring due to the intense competition especially below ground for the limiting resources of soil moisture and nutrients. This population is likely to be very competitive to newly establishing seedlings of any species and in particular *P. aquatica* seedlings because of the intensity of intraspecific competition. In hindsight, a more degraded *P. aquatica* pasture may have been a more suitable site for these experiments which could have resulted in better survival of emerged seedlings beyond spring through to summer.

Acknowledgements

The authors would like to thank Aaron Simmons and Warwick Badgery for commenting on an earlier draft of the manuscript, and Helen Nicol for statistical advice.

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Table 1 General Linear Regression (quadratic model) equations predicting seedling numbers across UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments with significant relationships; where SN = √ (seedling number m^{-2} + 0.5); P = *Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; C3 = C3 functional group DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; G = green DM t ha^{-1}; L = litter DM t ha^{-1}; PC = plant cover %; B = bare ground %, at emergence in March 2006 (Experiment 1) for *Phalaris aquatica* seedlings; the most significant factor was regressed against the number of seedlings (transformed) in Fig. 1.

Fig. 1 *Phalaris aquatica* seedlings m^{-2} (√ (n + 0.5) transformed) at emergence in March 2006 (Experiment 1) compared to significant factors (P = *Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; C3 = C3 functional group DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; G = green DM t ha^{-1}; PC = plant cover %; B = bare ground %; litter DM t ha^{-1}) of General Linear Regression (GLR) in Table 1; the 3 lines represent the most significant factor in the regression model with other factors set at median (or mean when median is 0; solid line) and mean ± 1 s.d. (dashed lines); equation numbers from Table 1 correspond to individual graphs; graphs 1-5 are UC (uncut), graphs 6-10 are CL (cut & leave) and graphs 11-17 are CR (cut & remove).

Fig. 2 *Phalaris aquatica* seedlings (m^{-2}) (a) at emergence in March 2006 (Experiment 1) as predicted by standing biomass DM t ha^{-1} and plant cover %, (b) at 24 weeks after emergence in August 2006 (Experiment 1) as predicted by green biomass DM t ha^{-1} and (c) at emergence in March 2007 (Experiment 2) as predicted by standing biomass DM t ha^{-1} across all treatments; standing DM t ha^{-1}, functional group DM t ha^{-1}, green DM t ha^{-1}, litter DM t ha^{-1}, bare ground %, plant cover % were initially included in the model.

Table 2 General Linear Regression (quadratic model) equations predicting seedling numbers across UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments with significant relationships; where SN = √ (seedling number m^{-2} + 0.5); P = *Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; G = green DM t ha^{-1}; L = litter DM t ha^{-1}; PC = plant cover %; B = bare ground %, at emergence in March 2007 (Experiment 2) for *Phalaris aquatica* seedlings; the most significant factor was regressed against the number of seedlings (transformed) in Fig. 3.

Fig. 3 *Phalaris aquatica* seedlings m^{-2} (√ (n + 0.5) transformed) at emergence in March 2007 (Experiment 2) compared to significant factors (*Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; G = green DM t ha^{-1}; L = litter DM t ha^{-1}; plant cover %; bare ground %) of General Linear Regression (GLR) in Table 2; the 3 lines represent the most significant factor in the regression model with other factors set at median (or mean when median is 0; solid line) and mean ± 1 s.d. (dashed lines); equation numbers from Table 2 correspond to individual graphs; graphs 1-4 are UC (uncut), graphs 5-10 are CL (cut & leave) and graphs 11-17 are CR (cut & remove).

Table 3 General Linear Regression (quadratic model) equations predicting seedling numbers across UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments with significant relationships; where SN = √ (seedling number m^{-2} + 0.5); P = *Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; G = green DM t ha^{-1}; L = litter DM t ha^{-1}; PC = plant cover %; B = bare ground %, at 24 weeks after emergence in August 2006 (Experiment 1) for surviving *Phalaris aquatica* young plants; the most significant factor was regressed against the number of seedlings (transformed) in Fig. 4.

Fig. 4 Surviving *Phalaris aquatica* young plants m^{-2} ($\sqrt{}$ (n + 0.5) transformed) at 24 weeks after emergence in August 2006 (Experiment 1) compared to significant factors (*Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; L = litter DM t ha^{-1}; B = bare ground %; others functional group DM t ha^{-1}; green DM t ha^{-1}; plant cover %;) of General Linear Regression (GLR) in Table 3; the 3 lines represent the most significant factor in the regression model with other factors set at median (or mean when median is 0; solid line) and mean ± 1 s.d. (dashed lines); equation numbers from Table 3 correspond to individual graphs; graphs 1-4 are UC (uncut), graphs 5-9 are CL (cut & leave) and graphs 10-13 are CR (cut & remove).

Table 4 General Linear Regression (quadratic model) equations predicting surviving *Phalaris aquatica* seedling numbers across UC (uncut), CL (cut & leave), CR (cut & remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify & rake) treatments with significant relationships; where SN = $\sqrt{}$ (seedling number m^{-2} + 0.5); P = *Phalaris aquatica* DM t ha^{-1}; AG = annual grasses DM t ha^{-1}; C3 = C3 functional group DM t ha^{-1}; OTH = others functional group DM t ha^{-1}; L = litter DM t ha^{-1}; PC = plant cover %; the most significant factor was regressed against the number of seedlings (transformed) in Fig. 5.

Fig. 5 Surviving *Phalaris aquatica* young plants m^{-2} ($\sqrt{}$ (n + 0.5) transformed) compared to significant factors (*Phalaris aquatica* DM t ha^{-1}; C3 functional group DM t ha^{-1}; others functional group DM t ha^{-1}; plant cover %; litter DM t ha^{-1}) of General Linear Regression (GLR) in Table 4; the solid line represents the most significant factor in the regression model; weak quadratic relationship not shown in graphs 2, 6 and 8; equation numbers from Table 4 correspond to individual graphs.

Table 1

Treatment			No	Equation	Adj R^2	P-value
UC	NS	NP	1	$SN = -45.4 + 2.43PC - 0.019PC^2 + 1.102B - 0.023B^2$	0.69	< 0.001
		HA	2	$SN = 34.39 - 7.69G + 0.92G^2 - 64.47C3 + 38.22C3^2$	0.83	< 0.001
	IS	NP	3	$SN = 37.72 - 1.15PC + 0.018PC^2 - 17.57OTH + 6.63OTH^2$	0.77	< 0.001
	SA	NP	4	$SN = 5.02 - 0.92P + 0.59P^2 + 20.06AG - 2.88AG^2$	0.69	< 0.001
		SR	5	$SN = 25 - 168.6AG + 580AG^2$	0.38	0.02
CL	NS	SR	6	$SN = 9.3 - 0.64L - 1.154L^2 + 61.7OTH - 196.2OTH^2$	0.48	< 0.001
	IS	NP	7	$SN = 8.4 - 52.2G + 27.34G^2 + 0.57PC + 0.0073PC^2 + 91OTH - 271OTH^2$	0.82	< 0.001
	SA	NP	8	$SN = 36.3 - 31.1G + 14.24G^2 + 37.3OTH - 22OTH^2 - 22P + 5.46P^2 -$	0.64	< 0.001
		HA	9	$SN = -26.78 + 3.15PC - 0.055PC^2 - 6.03G - 3.6G^2$	0.46	< 0.001
		SR	10	$SN = 4.49 + 8.18OTH - 1.02OTH^2 + 149.4C3 - 595C3^2$	0.72	< 0.001
CR	NS	NP	11	$SN = 2.7 - 4.3P + 1.85P^2$	0.40	< 0.001
		SR	12	$SN = 5.85 + 14.43P – 2.96P^2 - 4.04PC + 0.26 PC^2$	0.45	< 0.001
	IS	NP	13	$SN = -4.67 + 0.66PC - 0.0087PC^2$	0.34	< 0.001
		HA	14	$SN = 97.7 - 35.3L + 2.67L^2 - 0.24P + 1.51P^2$	0.78	< 0.001
	SA	NP	15	$SN = 20.5 + 0.2L - 0.977L^2$	0.25	0.004
		HA	16	$SN = 1.37 + 8.77G - 0.62G^2$	0.21	0.007
		SR	17	$SN = 17.31 - 24.8P + 10.51P^2$	0.36	< 0.001
Overall				$SN = -5.2 + 6.19P - 0.28P^2 + 0.17PC + 0.0024PC^2 - 13.5G + 2.58G^2 + 1.35OTH + 0.72OTH^2 + 0.38B - 0.0043B^2 - 3.54AG + 7.17AG^2$	0.52	< 0.001

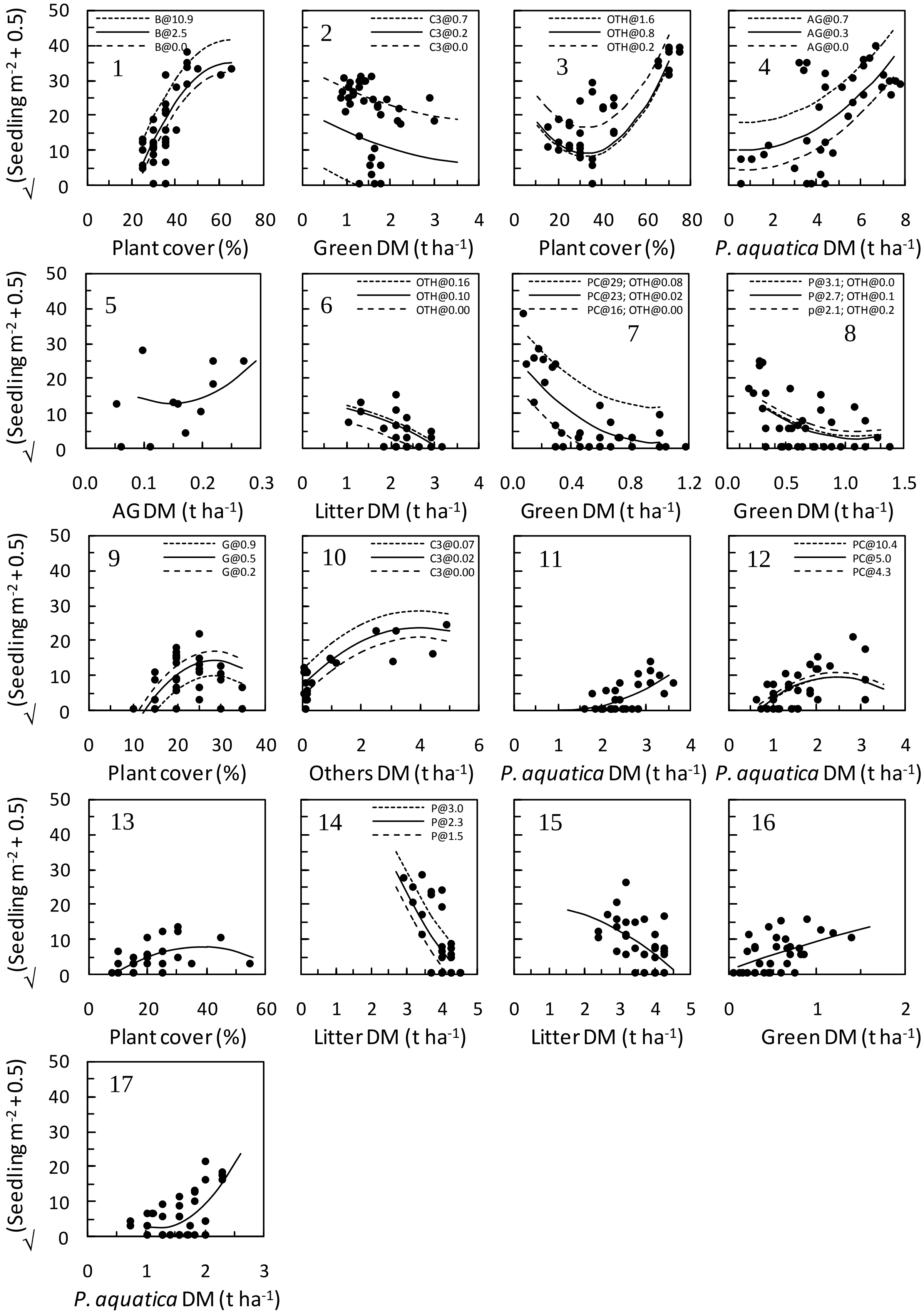

√(Seedling m⁻² + 0.5)
1
B@10.9
B@2.5
B@0.0
Plant cover (%)
2
C3@0.7
C3@0.2
C3@0.0
Green DM (t ha⁻¹)
3
OTH@1.6
OTH@0.8
OTH@0.2
Plant cover (%)
4
AG@0.7
AG@0.3
AG@0.0
P. aquatica DM (t ha⁻¹)
5
AG DM (t ha⁻¹)
6
OTH@0.16
OTH@0.10
OTH@0.00
Litter DM (t ha⁻¹)
7
PC@29; OTH@0.08
PC@23; OTH@0.02
PC@16; OTH@0.00
Green DM (t ha⁻¹)
8
P@3.1; OTH@0.0
P@2.7; OTH@0.1
p@2.1; OTH@0.2
Green DM (t ha⁻¹)
9
G@0.9
G@0.5
G@0.2
Plant cover (%)
10
C3@0.07
C3@0.02
C3@0.00
Others DM (t ha⁻¹)
11
P. aquatica DM (t ha⁻¹)
12
PC@10.4
PC@5.0
PC@4.3
P. aquatica DM (t ha⁻¹)
13
Plant cover (%)
14
P@3.0
P@2.3
P@1.5
Litter DM (t ha⁻¹)
15
Litter DM (t ha⁻¹)
16
Green DM (t ha⁻¹)
17
P. aquatica DM (t ha⁻¹)

Fig. 1

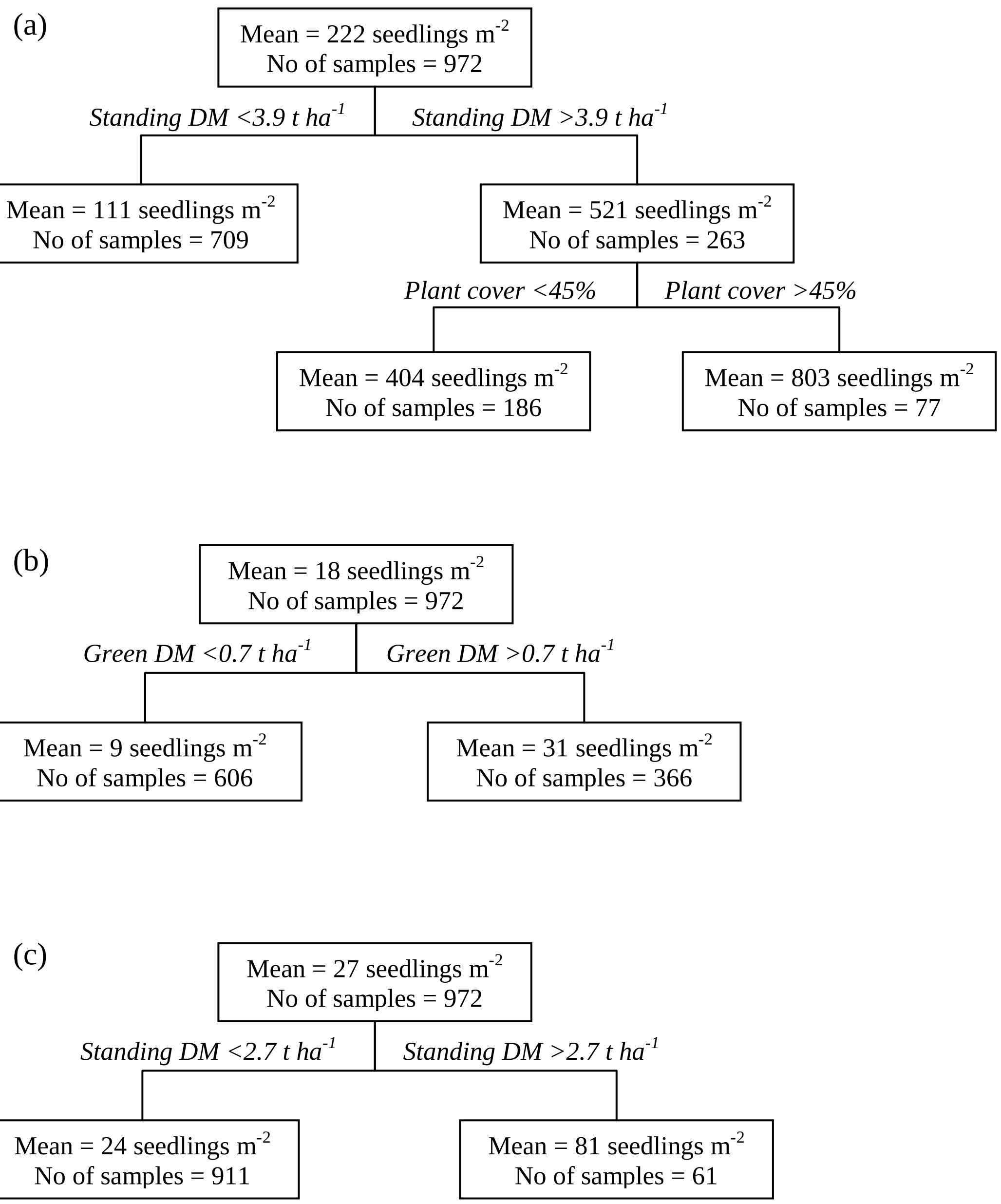

(a)
Mean = 222 seedlings m-2
No of samples = 972
Standing DM <3.9 t ha-1
Standing DM >3.9 t ha-1
Mean = 111 seedlings m-2
No of samples = 709
Mean = 521 seedlings m-2
No of samples = 263
Plant cover <45%
Plant cover >45%
Mean = 404 seedlings m-2
No of samples = 186
Mean = 803 seedlings m-2
No of samples = 77
(b)
Mean = 18 seedlings m-2
No of samples = 972
Green DM <0.7 t ha-1
Green DM >0.7 t ha-1
Mean = 9 seedlings m-2
No of samples = 606
Mean = 31 seedlings m-2
No of samples = 366
(c)
Mean = 27 seedlings m-2
No of samples = 972
Standing DM <2.7 t ha-1
Standing DM >2.7 t ha-1
Mean = 24 seedlings m-2
No of samples = 911
Mean = 81 seedlings m-2
No of samples = 61

Fig 2

Table 2

Treatment			No	Equation	Adj R^2	P-value
UC	NS	HA	1	$SN = 1.92 - 3.32P + 1.7P^2$	0.77	< 0.001
	IS	HA	2	$SN = 31.6 - 10L - 5L^2$	0.47	< 0.001
		SR	3	$SN = 28.98 - 20.72L$	0.14	0.02
	SA	NP	4	$SN = 13.59 - 0.99PC + 0.022PC^2$	0.43	< 0.001
CL	NS	NP	5	$SN = 1.59 - 5.67P + 7.55P^2$	0.21	0.009
		SR	6	$SN = 1.18 - 5.62P + 8.24P^2 - 8.02OTH + 27.54OTH^2$	0.48	< 0.001
	IS	NP	7	$SN = 3.25 - 11.85P + 14.02P^2$	0.24	0.004
		HA	8	$SN = 53.8 - 69.4L + 22.4L^2$	0.32	< 0.001
		SR	9	$SN = 9.74 - 1.96PC + 0.11PC^2$	0.20	0.01
	SA	NP	10	$SN = - 2.06 - 1.24PC + 0.084PC^2 + 45G - 61.9G^2$	0.41	< 0.001
CR	NS	NP	11	$SN = 148.2 - 181.9L + 55.9L^2$	0.41	< 0.001
		HA	12	$SN = 29.9 - 2.23P + 6.13P^2 - 39.9L + 13.16L^2$	0.36	0.001
	IS	NP	13	$SN = 1.08 - 3.42P + 7.38P^2$	0.29	0.001
		HA	14	$SN = 1.39 + 0.72B - 0.029B^2$	0.21	0.008
		SR	15	$SN = 5.62 - 17.58P + 27.17P^2 - 61.9AG$	0.39	< 0.001
	SA	SR	16	$SN = 35.7 - 66.5L + 32.7L^2$	0.25	0.004
		HA	17	$SN = - 11.23 + 1.32PC$	0.37	< 0.001
Overall				$SN = 2.25 + 3.26P - 0.62P^2 - 14.11AG + 12AG^2 + 0.2PC - 0.0017PC^2 - 12.51G + 9.79G^2$	0.18	< 0.001

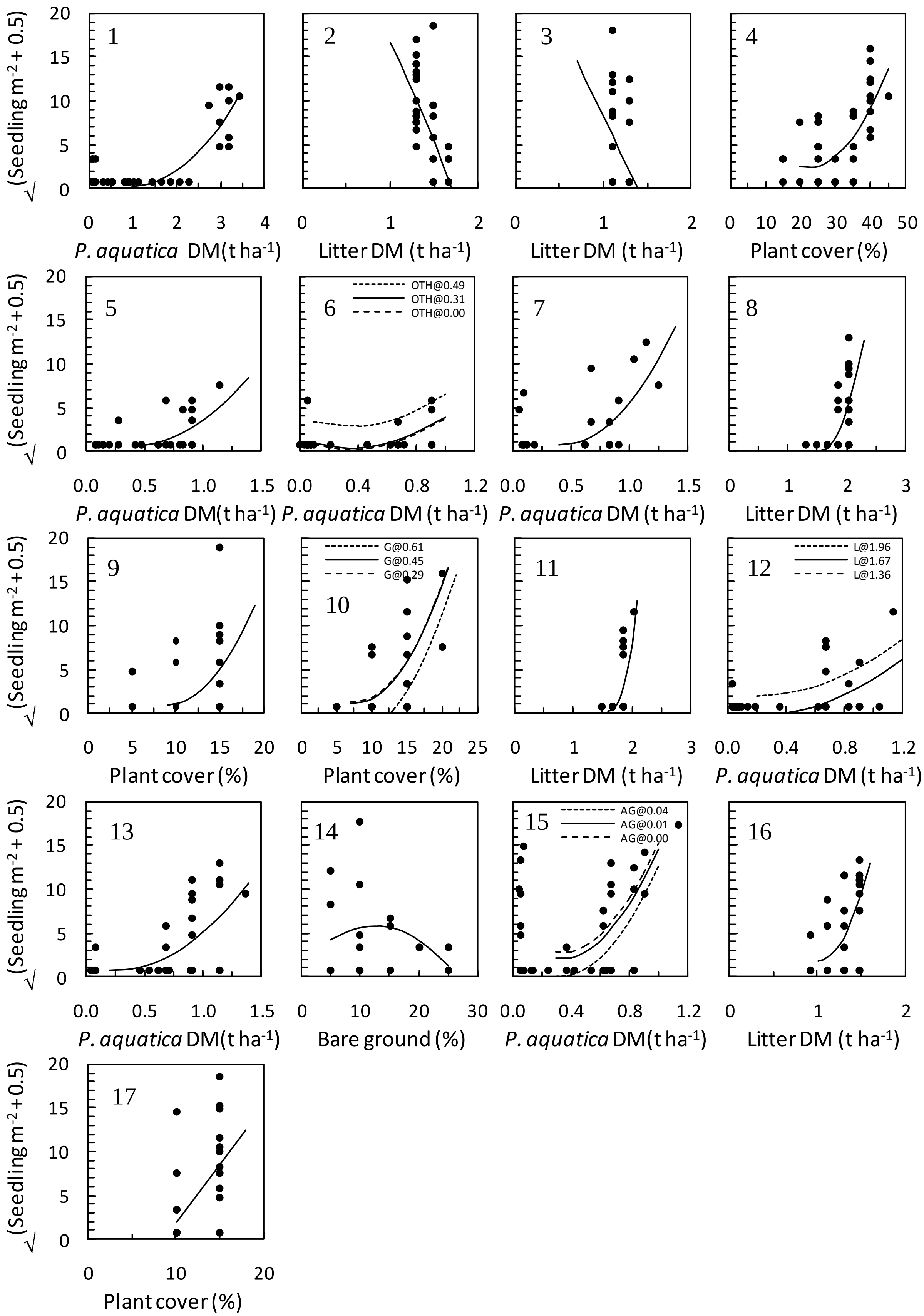
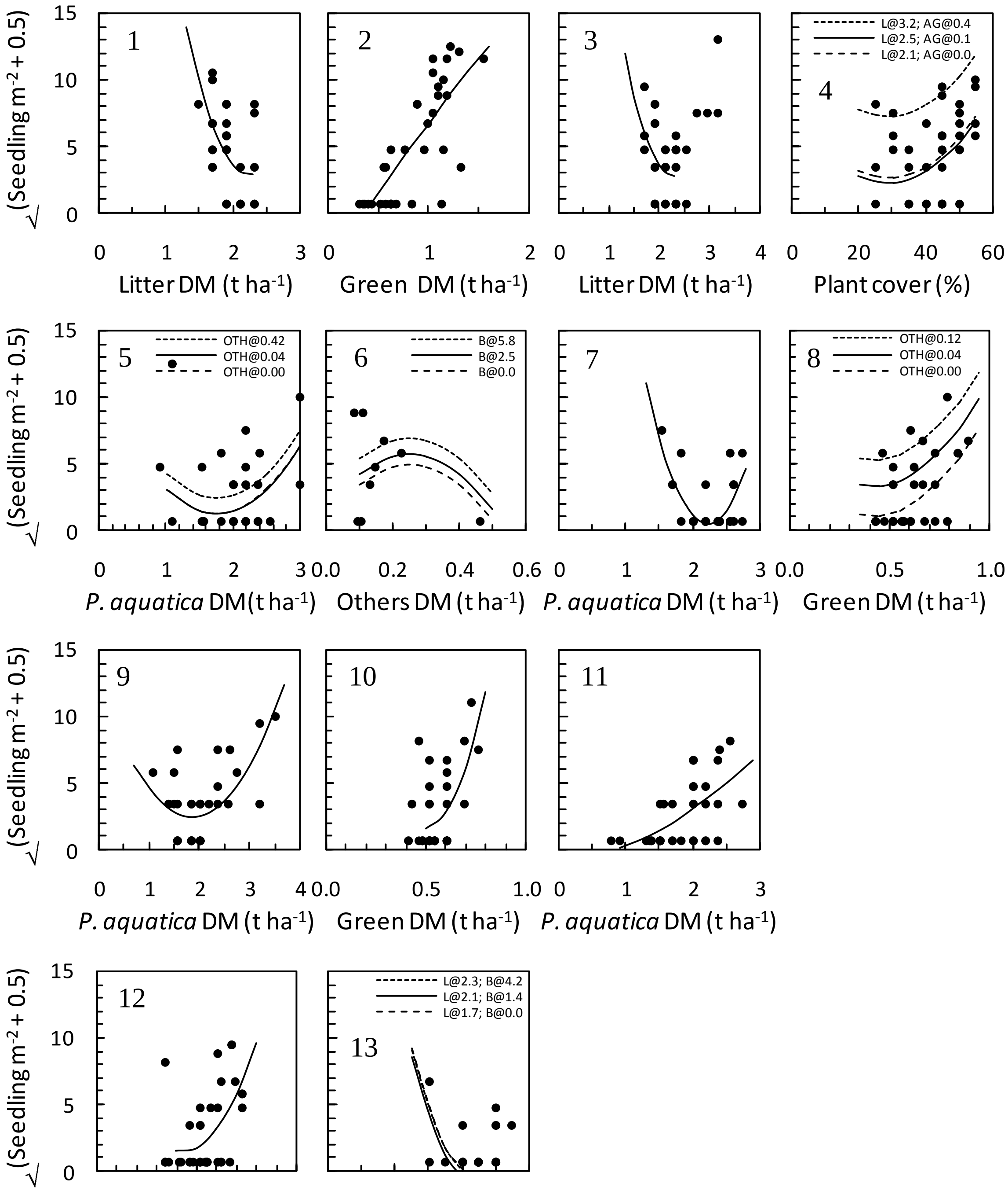

√(Seedling m-2 + 0.5)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
P. aquatica DM(t ha-1)
Litter DM (t ha-1)
Plant cover (%)
Bare ground (%)
OTH@0.49
OTH@0.31
OTH@0.00
G@0.61
G@0.45
G@0.29
L@1.96
L@1.67
L@1.36
AG@0.04
AG@0.01
AG@0.00

Fig. 3

Table 3

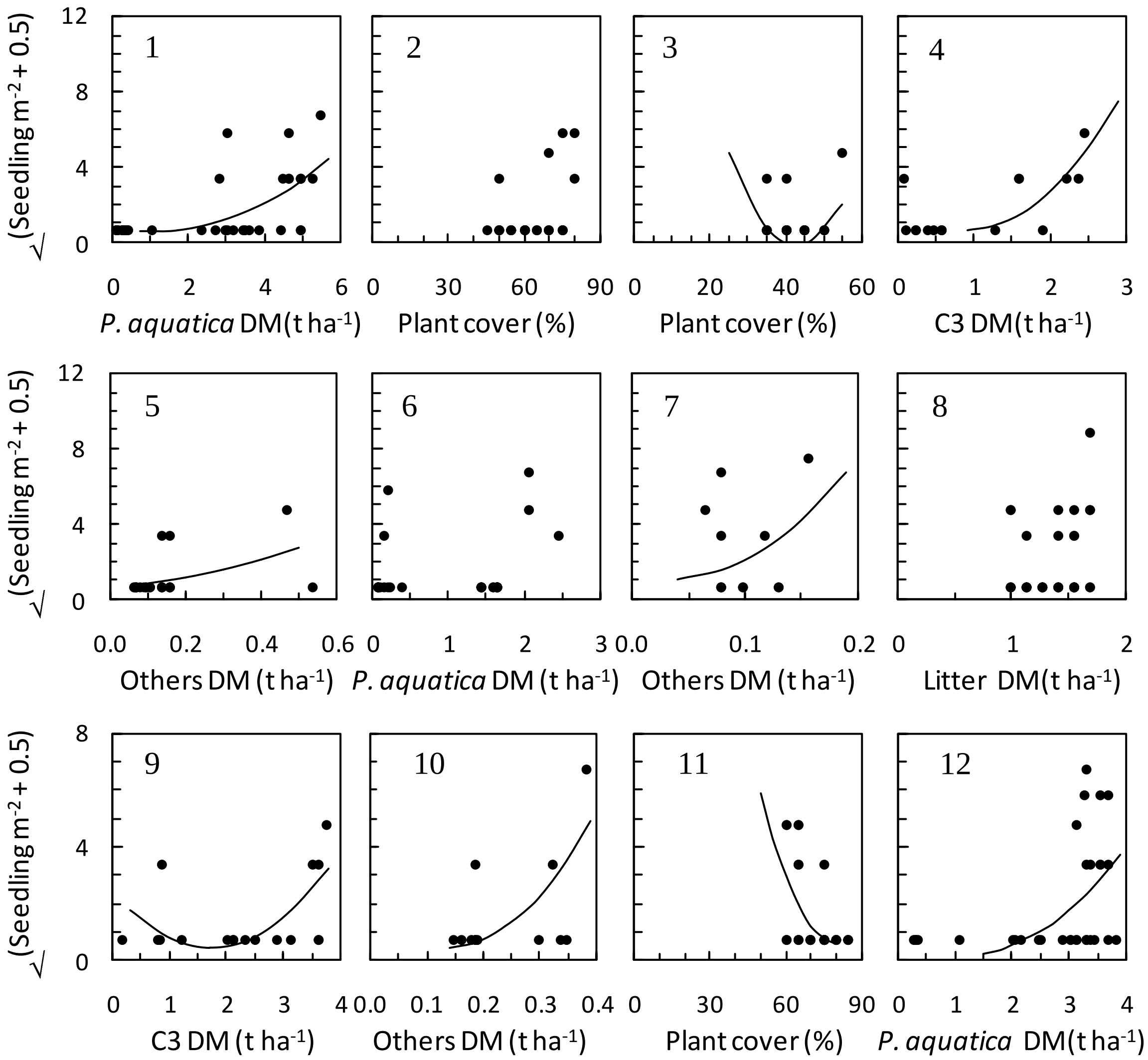
Treatment			No	Equation	Adj R^2	P-value
UC	NS	HA	1	$SN = 64 - 54.2L + 12.02L^2$	0.23	0.005
	SA	NP	2	$SN = - 4.17 + 11.81G - 0.86G^2$	0.59	< 0.001
		HA	3	$SN = 51.8 - 42.8L + 9.35L^2$	0.39	< 0.001
		SR	4	$SN = 36.8 - 0.38PC + 0.0066PC^2 - 22.79L + 4.32L^2 + 20.2AG - 22.4AG^2$	0.50	< 0.001
CL	NS	NP	5	$SN = 10.82 - 10.9P + 3.13P^2 - 0.98OTH + 8.91OTH^2$	0.57	< 0.001
		SR	6	$SN = 0.76 + 33.36OTH - 66.4OTH^2 + 0.31B + 0.0046B^2$	0.54	< 0.001
	IS	HA	7	$SN = 61.6 - 54.94P + 12.36P^2$	0.50	< 0.001
	SA	NP	8	$SN = 5.15 - 19.3G + 23.1G^2 + 67.2OTH - 270OTH^2$	0.38	< 0.001
		SR	9	$SN = 12.39 - 10.7P + 2.89P^2$	0.39	< 0.001
CR	NS	HA	10	$SN = 29.9 - 113.2G + 113.3G^2$	0.32	< 0.001
		SR	11	$SN = - 0.87 + 0.46P + 0.75P^2$	0.30	< 0.001
	IS	HA	12	$SN = 12.91 - 52.8G + 60.9G^2$	0.25	0.003
		SR	13	$SN = 84 - 2.36PC + 0.027PC^2 - 33.96L + 8.63L^2 + 0.047B - 0.043B^2$	0.59	< 0.001
			Overall	$SN = 9.79 + 7.61G - 2.62G^2 - 11.45L + 2.76L^2 + 5.22OTH - 1.95OTH^2 + 0.24P + 0.004P^2$	0.26	< 0.001


√(Seedling m-2 + 0.5)
1
Litter DM (t ha-1)
2
Green DM (t ha-1)
3
Litter DM (t ha-1)
4
L@3.2; AG@0.4
L@2.5; AG@0.1
L@2.1; AG@0.0
Plant cover (%)
5
OTH@0.42
OTH@0.04
OTH@0.00
P. aquatica DM(t ha-1)
6
B@5.8
B@2.5
B@0.0
Others DM (t ha-1)
7
P. aquatica DM (t ha-1)
8
OTH@0.12
OTH@0.04
OTH@0.00
Green DM (t ha-1)
9
P. aquatica DM (t ha-1)
10
Green DM (t ha-1)
11
P. aquatica DM (t ha-1)
12
Green DM (t ha-1)
13
L@2.3; B@4.2
L@2.1; B@1.4
L@1.7; B@0.0
Plant cover (%)

1 Fig. 4

Table 4

Treatment			No	Equation	Adj R^2	P-value
At 24 weeks after emergence (Experiment 2 - August 2007)						
UC	NS	NP	1	$SN = 0.82 - 0.4P + 0.18P^2$	0.37	< 0.001
		HA	2	$SN = 21.69 - 0.73PC + 0.0063PC^2$	0.30	0.001
		SR	3	$SN = 27.58 - 1.29PC + 0.015PC^2$	0.29	0.001
	SA	SR	4	$SN = 2.25 - 3.29C3 + 1.76C3^2$	0.15	0.03
CL	NS	SR	5	$SN = 0.69 + 1.41OTH + 5.35OTH^2$	0.22	0.006
	IS	NP	6	$SN = 2.14 - 4.39P + 2.32P^2$	0.28	0.002
	SA	NP	7	$SN = 1.44 - 17.1OTH + 237OTH^2$	0.19	0.01
CR	IS	HA	8	$SN = 26.1 - 39.8L + 15.74L^2$	0.16	0.02
		Overall		$SN = 1.65 + 0.17P + 0.0035P^2 - 2.63AG - 2.71AG^2 - 0.62C3 + 0.2C3^2$	0.08	< 0.001
At 52 weeks after emergence (Experiment 2 - March 2008)						
UC	IS	NP	9	$SN = 2.4 - 2.22C3 + 0.64C3^2$	0.18	0.01
	SA	SR	10	$SN = 1.61 - 18.04OTH + 67.8OTH^2$	0.19	0.01
CR	IS	HA	11	$SN = 37.9 - 0.93PC + 0.0058PC^2$	0.25	0.003
	SA	HA	12	$SN = 0.86 - 1.1P + 0.47P^2$	0.22	0.006
		Overall		$SN = 1.083 - 0.06P + 0.062P^2$	0.03	< 0.001



√(Seedling m-2 + 0.5)
1
2
3
4
5
6
7
8
9
10
11
12
P. aquatica DM(t ha-1)
Plant cover (%)
Plant cover (%)
C3 DM(t ha-1)
Others DM (t ha-1)
P. aquatica DM (t ha-1)
Others DM (t ha-1)
Litter DM(t ha-1)
C3 DM (t ha-1)
Others DM (t ha-1)
Plant cover (%)
P. aquatica DM(t ha-1)

1 Fig. 5

Influence of depth and time of sowing on the establishment of warm-season perennial pastures in the agricultural regions of Western Australia.

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Abstract

Two studies were conducted in south west Western Australia to examine some of the factors affecting the successful emergence of seedlings of five species of sub-tropical perennial grasses [*Megathyrsus maximus* cv. Gatton (panic), *Urochloa decumbens* cv. Basilisk (signal), *Chloris gayana* cv. Katambora (Rhodes grass), *Pennisetum clandestinum* cv. Whittet (Kikuyu) and *Setaria splendida* cv. Splendid)] and the perennial legume (*Lotononis bainesii* cv. Miles). The first experiment investigated the effects of depth and two times (month) of sowing on sub-tropical perennial pasture seedling emergence, while a second study examined the effects on time of sowing at weekly intervals on seedling emergence. The trials has shown that a single sowing depth of 5-10 mm appears suitable for each of Gatton panic, Rhodes grass, signal grass, kikuyu, *Setaria splendida* and *Lotononis bainesii*, while surface-sown seed of all species failed to establish. Although the seeds on the surface were not pressed in to 1-2mm, the data indicates that there is enhanced establishment with burial. The trial displayed that the time of sowing had no effect on seedling establishment, but earlier sowing produced more biomass of all species, with the exception of *Setaria splendida*. In early August The emerged seedlings were visually stressed from the cool conditions and have subsequently

had to compete with many annual weeds. It was also observed that of the 100 germinable seeds sown, not even 30% of these seedlings survived to 20 days after sowing. Seedling survival was highest (100%) when seed was sown on the 15th August, although reasonable survival occurred throughout the August sowings. The September sowings produced the lowest seedling survival ranging from 68% at the beginning, to a low of 40% by the end of the month. Optimal weed control was achieved when the second knockdown occurred on or after the 8th August and optimal plant production was achieved from the seed sown on the 8th August, however high production was also attained in the sowing window 2 weeks before and after this date.

Introduction

Over the last decade warm season perennial grasses are attracting considerable farmer interest in both Western Australia and New South Wales for their potential out-of-season productivity and their ability to reduce groundwater recharge (Boschma *et al.* 2008, 2009; Lodge 2010). They have been widely used in the northern slopes and plains of NSW and there is increasing interest in the northern agricultural and south coast regions of WA, largely driven by farmer groups such as Evergreen (Moore *et al.* 2006). However, focus-group studies indicated that more information was required to enable producers to reliably establish these grasses (McCormick *et al*. 2009). In the agricultural regions of WA the optimum sowing window in spring is narrow, due to the need for warm soil temperatures for germination conflicting with decreasing rainfall reliability. This results in plants trying to establish in drying soil profiles. A further challenge is the sand-based texture of the majority of soils in the WA target zones for warm season perennials. These soils are typically water repellent and prone to rapid drying in spring. Additionally, studies of tropical perennial grass persistence (Moore *et al*. 2006) in Western Australia (WA), also reported poor persistence over the first winter as a result of a combination of cold, wet soils and frosts. In both of these studies, Rhodes grasses (*Chloris gayana*) had good winter survival, while some *Panicum* spp. (both studies) and digit grass (*Digitaria eriantha* ssp. *eriantha* cv. Premier, WA study, Kojunup site) had lower survival. Cold weather particularly frosts are a similar problem that occur in the North-West Slopes of

NSW (Hobbs and Jackson 1977) and south-eastern Queensland (Jones 1969) where high mortality of tropical (C4) grasses are attributed to frequent and severe frosts in the first winter.

Measurements taken in 2005 showed establishment densities for commercial warm season perennial grass sowings in Western Australia typically result in about 1 plant/m^2 for each kg of seed sown per ha (Moore et al. 2006). Assuming a seed viability of 50%, this gives a seedling establishment of only 1-2%. This low plant density is a major impediment to the potential production from these sub-tropical grass pastures, especially for the bunch grasses which usually display very low plant recruitment from seed.

There is thus enormous scope for increasing establishment density and therefore, the potential production of warm season grass pastures in Western Australia. Higher establishment densities will allow a reduction in seeding rates, reducing establishment costs and hopefully increasing adoption. As researchers, our challenge is to develop and provide a robust and reliable establishment package that gives consistently good results. However in general, the conditions for successful establishment of exotic warm season perennials are often not well understood by farmers and are not well underpinned by science.

A new project titled “Reliable establishment of non-traditional perennial pasture species”, funded through the Salinity CRC (with partners including MLA, AWI and SGSL), is conducting research into improving establishment of a range of perennial species. This paper describes our preliminary investigations into the influence of depth and time of sowing of six exotic warm season perennials.

Materials and Methods

A micro-plot field trial was conducted on a sandy soil at South Perth under irrigation to determine the optimum sowing depth of six exotic warm-season perennial species. The trial consisted of 5 perennial grasses (Gatton panic, Katambora Rhodes grass, Signal grass, Whittet Kikuyu, and Splenda Setaria) and the perennial legume (*Lotononis bainesii* cv. Miles) sown at two dates (24 August and 28 September, 2006). The experiment comprised 288 plots sown to 100 germinable seeds placed in 400mm row lengths at six different depths (surface (without pressing), 5 mm, 10mm, 15mm, 20mm, and 30mm) and replicated four times. Depth was controlled by a wooden dowel hammered precisely to the required depths, seeds placed at the bottom and filled by red sand to the surface. Emerged seedlings were counted weekly for 28 days. Two randomly chosen seedlings per plot were grown and harvested on 2 December for measurement of dry weight.

A time of sowing trial was recently conducted in 2007 on a sandy duplex soil at Gillingarra, WA (150 km NNE of Perth) to sort out this conundrum. This region has a Mediterranean climate with a winter dominant rainfall (85%) and average annual rainfall of approximately 480 mm.

Gatton panic, Rhodes grass and the perennial legume lotononis (*Lotononis bainesii)* were hand sown on weekly intervals for 10 weeks from 25th July to 26th September. The trial area was initially sprayed with 2L/ha glyphosate on 18th July and again one week prior to each sowing. This routine was repeated until the 6th sowing time on the 29th August when all the remaining plots were sprayed (Table 1). The site received only 130 mm rainfall from July to September which is below the average.

Seedling emergence and survival, and plant production for each sowing date were compared. In addition, the weed burden of the plots was assessed and soil temperature recorded.

Results

Results indicate that seed sown on the surface of all species failed to establish, while seed of all species established well from 5 mm and 10 mm sowing depth. Whereas, the smaller-seeded species had major reductions in establishment density at deeper sowing depths, the larger-seeded species (eg. signal grass) were able to establish from deeper sowings. Notably, establishment of Rhodes grass was severely affected at depths under 10mm. The important finding from this experiment is that a single sowing depth of 5-10 mm appears suitable for each of the species studied. The time of sowing (TOS) did not have a significant effect on germinating seedling numbers. However, the TOS had a highly significant impact on biomass production. The first TOS had greater biomass production in comparison to the second TOS, with the exception of Setaria.

Large differences in the establishment of the perennial pastures were observed. Surprisingly, the maximum soil temperature rose above 15°C in the week following the first time of sowing in late July and the seeds germinated. However, the newly emerged seedlings were visually stressed from the cool conditions and have subsequently had to compete with many annual weeds. The results suggest that the key factor relates to the number of hours when the soil temperature is above 15°C.

It was also observed that of the 100 germinable seeds sown, not even 30% of these seedlings survived to 20 days after sowing. However, seedling survival was highest (100%) when seed was sown on the 15th August, although reasonable survival occurred throughout the August sowings (Figure 1). The September sowings produced the lowest seedling survival ranging from 68% at the beginning, to a low of 40% by the end of the month.

The best weed control was achieved when the second knockdown occurred on or after the 8th August. Additionally, by the end of October, optimal plant production was achieved from the seed sown on the 8th August, however high production was also attained in the sowing window 2 weeks before and after this date (Table 1).

Discussion

This trial has shown that a single sowing depth of 5-10 mm appears suitable for each of Gatton panic, Rhodes grass, signal grass, kikuyu, *Setaria splendida* and *Lotononis* *bainesii*, while surface-sown seed of all species failed to establish. Although the seeds on the surface were not pressed in to 1-2mm, the data indicates that there is enhanced establishment with burial. The trial displayed that the time of sowing had no effect on seedling establishment, but earlier sowing produced more biomass of all species, with the exception of *Setaria splendida.*

Researchers at the Department of Agriculture and Food Western Australia are investigating the optimal sowing window for a range of warm-season perennial grasses and legumes. This is part of a project to develop reliable, robust and economical establishment packages for non-traditional species.

The current guidelines suggest sowing in early spring when the average soil temperature is above 15°C. Sowing time is always a compromise between sowing early when the soil temperature is marginal for germination and growth, versus the decreasing likelihood of good follow-up rainfall the longer seeding is delayed.

These results indicate the need to sow at a conservative speed and to check that seed is being consistently placed 5-10 mm below the surface. The machine was set up in this experiment to sow at this depth when speed was 5 km/hr. At faster sowing speeds greater soil disturbance indicates the machine no longer consistently sowed at this depth, resulting in less accurate seed placement and lower resultant plant densities. This may not mean that all sowing should be done at low speeds. However, if a faster sowing speed is to be used, it is important to set up the machine for sowing at that particular speed. This research also highlights the need to check seed placement before and during sowing warm season perennial grass paddocks.

Accurate seed placement appears critical for reliable establishment of sub-tropical grasses. A sowing depth of 5-10 mm is optimum for the sub-tropical species currently used, while use of press wheels provides good seed-soil contact. Sowing speed has an effect on distribution of soil behind the tynes; higher speed creating greater disturbance.

This experiment was undertaken to determine the effect of different sowing speed on establishment density, as part of a project to develop a best-practice method for reliable establishment of sub-tropical grasses.

Acknowledgements

This work was conducted through the CRC for Future Farm Industries with funding provided by Meat & Livestock Australia, Australian Wool Innovation and Land & Water Australia. John Titterington, Brad Wintle and Daniel Kidd provided excellent technical support.

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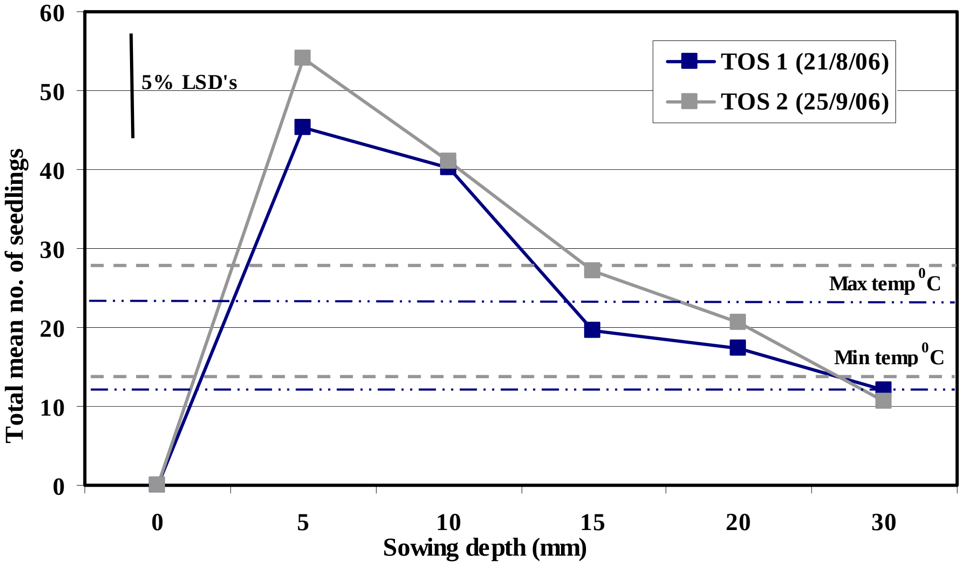

Figure 1. Mean number of seedlings established following two times of sowing (TOS) at six sowing depths (mean of six species).

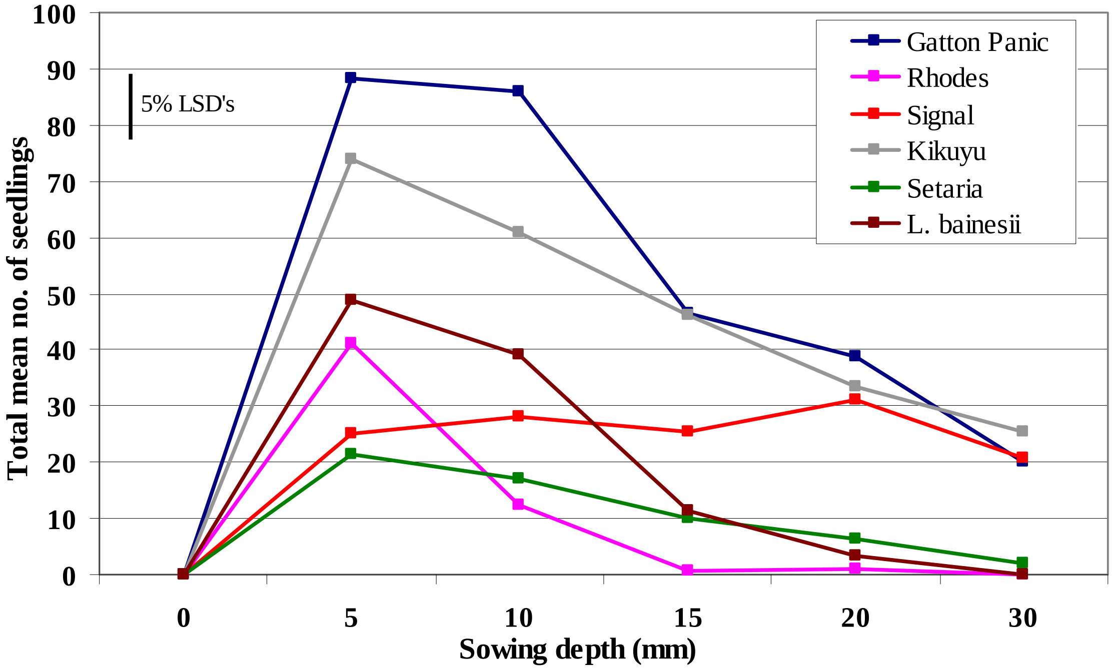

Figure 2. Mean number of seedlings established by six exotic warm-season perennial species at six sowing depths (mean of two sowing times)

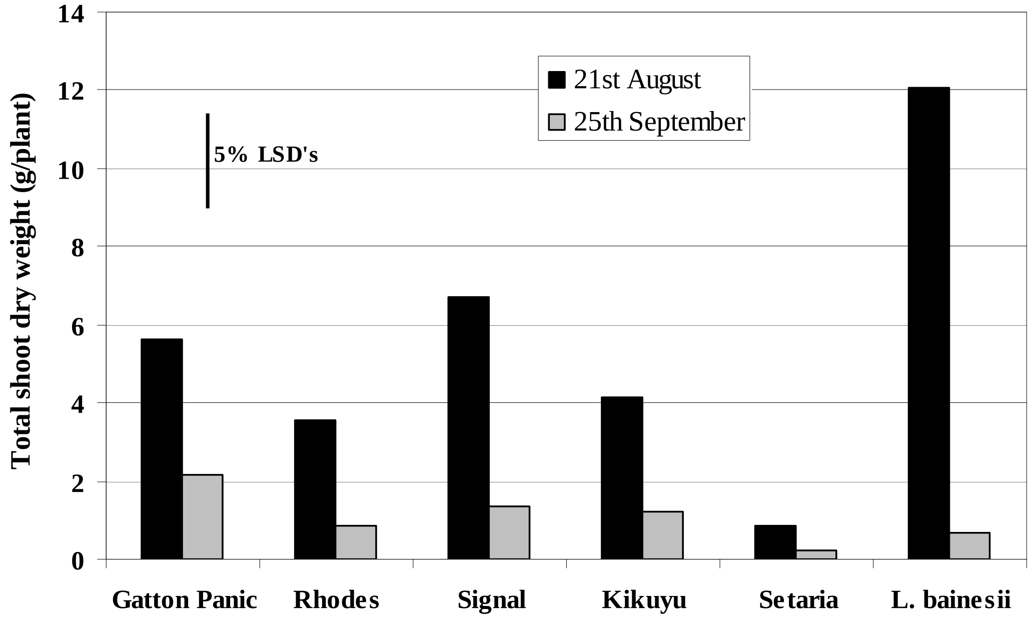

Figure 3. Mean dry matter production (g/plant) of six warm-season perennial species from two sowing dates.

Table 1. Combined measurements of seedlings (counts[#] and percentage survival) and plant production for three warm season perennials (Rhodes grass, lotononis and Gatton panic) plus the weed burden and soil temperature data with 10 weekly sowing times from the 25th July, 2007 at Gillingarra, WA. ([#] Germination from 100 germinable seeds, * Sown in to dry soil)

Sowing date	Second spray date	Weekly mean min-max soil temp (°C)	Weekly mean hours per day soil temp >15°C	Seedling counts (20 days) and % survival after 40 days	Weed burden	Plant prod. on 24/10/07
25th July	18th July	8.5 - 17	6	20 (80%)	very high	high
1st Aug	25th July	10 - 18.5	6.5	24 (96%)	high	high
8th Aug	1st Aug	10.5 - 17.5	7.5	24 (94%)	medium	very high
15th Aug	8th Aug	9 - 18.5	8	26 (100%)	low	high
22nd Aug	15th Aug	9 - 22.5	10	26 (81%)	low	high
29th Aug	22nd Aug	7 - 24	10.5	26 (85%)	low	medium
5th Sept	22nd Aug	9.5 - 25	10.5	29 (68%)	low	low
12th Sept	22nd Aug	8.5 -23.5	11.5	29 (54%)	low	low
19th Sept	22nd Aug	10 – 25.5	10.5	27 (57%)	low	low
26th Sept*	22nd Aug	13 -27	13	14 (40%)	low	very low

Plant signalling chemicals for enhanced germination and emergence in perennial pasture species

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Introduction

The germinability and hence emergence of species is regulated by an inherited seed dormancy state, temperature, moisture and light interactions. In previous experiments seed dormancy state in each species was identified, along with the role of temperature and moisture, in regulating germination. This was necessary to identify which species have physiological dormancy issues that will affect field emergence significantly as well as to determine the approximate sowing windows for species in the field, particularly those where there is a paucity of information relating to sowing time. Seed enabling treatments involving physical seed testa/capsule manipulation and use of hydro-priming to incorporate plant signalling chemicals into seeds were also identified to improve germinability in several species under controlled conditions. In particular, plant signalling chemicals were identified to significantly promote germination in otherwise physiologically dormant species such as *Megathyrsus maximus* cv. Gatton (Panic Grass), *Digitaria eriantha* cv. Premier (Digit Grass), *Maireana brevifolia* (Small Leaf Bluebush), *Austrostipa elegantissima* (Feather Speargrass), *Atriplex semibaccata* (Creeping Saltbush) and *Rhagodia preissii* (Rhagodia).

In non-dormant species, the efficacy of hydro-priming and plant signaling chemicals is often masked by the interactions between temperature and moisture. Thus, if the chemical enabling treatment is applied at a seed's optimal germination temperature and moisture requirement, the enabling effect observed will be minimal as the species germinates well regardless. As plant signaling chemicals act at a biochemical level to overcome germination regulatory mechanisms, testing enabling treatments in seeds placed at sub-optimal conditions, such as moisture stress, tends to clearly reduce the efficacy of a chemical or series of chemicals on germination. This testing scenario also simulates what a seed may experience under field conditions.

Kinetin and salicylic acid have been observed to enable germinating seedlings overcome short-term stressful conditions (Senaratna *et al.* 2003, Stevens *et al.* 2006) and may be useful in improving germination, emergence and survival of seedlings under field conditions. Kinetin is a cytokinin, a class of plant hormone that promotes cell division, shoot and root morphogenesis, chloroplast maturation, cell enlargement and auxiliary bud release and senescence. The function of this plant hormone requires that auxin is present. In contrast, salicylic acid is an inducer of systemic acquired resistance (SAR) to diseases in plants (Raskin 1992; Conrath *et al*. 1995) and is known to interact with plant respiration (Bourbouloux *et al*. 1998) and protein synthesis (Jin *et al*. 2000). Work by Dat *et al.* (1998) and Senaratna *et al.* (2003) shows that the use of salicylic acid increases thermo tolerance in seedlings and more recently, improves germination under moisture and saline stress in *Atriplex* species (Stevens *et al*, 2006). Unfortunately, the mode of action of these chemicals during germination remains unresolved. However, combined with other plant signaling chemicals, both kinetin and salicylic acid may provide additional support to improving germination, emergence and plant establishment.

In these set of experiments and trials, it was hypothesised that combining plant signaling chemicals with kinetin and or salicylic acid, improve germination and emergence in a range of project species. This improvement will be based on the degree of physiological dormancy in the species as well as the extent of moisture regulation on germination.

Materials and methods

Seeds of all species used in these experiments had been cleaned of inert material and dried for a minimum of 14 days at 16°C at 25% relative humidity. Seed for all experiments were >98% viable, confirmed using the tetrazolium staining method described by the International Seed Testing Association (1999).

Critical moisture stress to test the efficacy of plant signalling chemical combinations

Critical moisture stress for seed was defined as a water potential that retards germination by 75% of maximum germination. The effect of moisture stress on germination was investigated by supplementing germination media with polyethylene glycol (PEG, molecular weight 8000) equating to the solute potentials of 0,−0.25,−0.5,−0.75,−1, -1.25, −1.5 and −2MPa. The osmotic potentials were calculated by equation 1, where *x* is the concentration of PEG_{8000} by %w/v (Michel and Kaufmann 1973).

$$\Psi_{PEG8000}\,(\mathrm{MPa}) = -7.6049x2 - 33.025x + 4.83 \qquad (1)$$

Four replicates of 25 seeds of each species were plated into 90 mm vented peri dishes containing a filter paper with 8 mL of each solute potential. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20°C 12/12 hour light/dark constant incubator (TLMRIL model, Thermoline, QLD, Australia). This temperature was chosen as the osmolytic regression in equation (1) by Michel and Kaufmann (1973) and is correlated with temperature. Germination, defined as 1 mm radicle emergence followed by continued growth, was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index was calculated using equation 2 and 3. All germination data was arcsine transformed and an ANOVA was conducted to compare significance between treatments. Regressions and their equations in relation to the osmolytic gradient was plotted using CurveExpert 1.4 (Daniel Hyams, 2009). These equations were used to determine the critical moisture stress for each species at 20°C.

$$\text{Final germination \% at } t = [\Sigma G_t / r] \times 100 \qquad (2)$$

Where: t = incubation period (days);

G = total germinated seeds across treatment petri dishes;

r = number of replicates

Germination rate index (GRI) = $\sum[(D_i - D_{i-1})/_i]$, (3)

Where: I = is the germination count day;

D_i = the percentage of seeds germinated at time "i";

D_{i-1} is the percentage of seeds adjudged germinated the previous count day (from Maguire, 1962).

Efficacy of combined plant signaling chemical combinations (PSCC) at critical moisture stress

Once critical moisture stress had been determined, plant signaling chemicals (PSC) identified for each species were combined using either 0.05 mM kinetin (K) and or 0.5 mM salicylic acid (SA). The following treatments were prepared: no treatment (cnt), H_2O, PSC, K, SA, K+SA, PSC+K, PSC+SA, PSC+K+SA. Seeds were hydro-primed in treatments for 18 hours, extracted, rinsed under water and patted dry with a paper towel. Primed seeds were then left to bench dry for two days at 22^{o}C, 55% relative humidity, then transferred to a 16^{o}C, 25% relative humidity room to further dry for another four days. Four replicates of 25 seeds of each treatment were plated into 90 mm vented Petri dishes containing a filter paper to which 8 mL of the critical moisture stress solute potential for each species was added. Controls consisted of treated seed plated into Petri dishes with filter paper containing only water. Petri dishes were subsequently sealed with plastic to retard evaporation and placed into a 20^{o}C dark constant incubator (TLMRIL model, Thermoline, QLD, Australia).

Germination was scored regularly over a 14 day incubation duration. Final germination percentage and germination rate index across treatments were calculated using equation 2 and 3. All germination data was arcsine transformed and an ANOVA was conducted to compare significance between treatments. This enabled identification of optimum plant signaling chemical combinations (PSCC) for glasshouse and field trials.

Efficacy of combined plant signalling chemicals in glasshouse and field conditions

The majority of laboratory, glasshouse and field trials were set up over the 10-19 Sept 2008. However, glasshouse trials for chenopod species were conducted from 21 May 2009. Three sets of treated seeds were prepared: for laboratory controls; for glasshouse trials; and for field trials.

The following treatments were prepared: no treatment (cnt), H_2O and PSCC. For glasshouse trials, four replicates of 50 seeds of each treatment were sown at 5-7 mm depth into 150 mm diameter pots containing free draining white sand to a 140 mm depth. Pots were irrigated daily and emergence was scored weekly over a 120 day period.

A free-draining sandy site was selected at the University of Western Australia Shenton Park Field Station for field trials. Prior to seeding, the site was rotary hoed and received two applications of glyphosate at 2 L/ha (a.i. 680 g/L), three weeks and two weeks prior to sowing. The site also received 150 kg/ha NPK fertiliser four weeks prior to sowing. Seeding commenced on 18 September 2008. The seed of each treatment was hand sown into 1 m long rows, 15 cm apart, to a depth of 7-9 mm. The trial had a latin square design with four replicates. Pyrethrin was applied one week post-sowing for insect control. Plots were scored weekly for 30 days, then fortnightly over a 120 day period.

The laboratory controls were run on the 10 September 2008 using the laboratory germination methodology described. However, an alternating 26/13^{o}C 12/12 hour temperature was used and seeds were plated on 0.6% water agar impregnated with 0.1% plant preservation mixture. Treatments were incubated in the dark.

Results

Critical moisture stress to test the efficacy of plant signalling chemical combinations

Seven plant signalling chemical combinations were identified to be significantly effective in improving germination, using moisture stress to differentiate treatments (Table 2). These were GA3+SA for *A. semibaccata*, KAR1+SA for *A. bunburyana*, GA3+K+SA for *M. brevifolia,* GA3+SA+K for *R. preissii*, *Ethy+SA* for *M. maximus*, KAR+SA+K for *S. sphacelata* and KAR1 for *M. atropurpureum*. Chemical combinations listed for *P. ciliata, T. ponticum, M. sativa, P. clandestinum, C. gayana, D. eriantha* and *A. saligna* were on average higher than controls but not significant. Plant signalling chemical combinations were highly significant in three species. Under critical moisture stress conditions GA3+SA improved germination in *A. semibacatta* from 0% (±0) to 20% (±2.3%), KAR1+SA improved germination in *A. bunburyana* from 6% (±1.3) to 24% (±3.3%) and GA3+SA+K improved germination in R. preissii from 4% (±1.9) to 29% (±4.9%).

Efficacy of combined plant signalling chemicals in glasshouse and field conditions

Under laboratory conditions, plant signaling chemical combinations improved germination significantly to controls in *A. bunburyana, M. brevifolia, P. ciliata, R. preissii*, *M. maximus, C. gayana, S. sphacelata, D. eriantha* and *A. saligna* (Table 3). Under glasshouse conditions, chemical combinations improved emergence significantly after 30 days in *M. brevifolia*, *A. semibaccata*, *R. preissii*, *M. maximus*, *P. clandestinum*, *D*. *eriantha*, *S. sphacelata, M. atropurpureum* and *A. saligna*. However, there was a general lack of transfer of the successful laboratory and glasshouse treatments to the field environment. Under field conditions, only *R. preissii, M. maximus, D. eriantha* and *A. saligna* showed significant improvements compared with controls in 30-day emergence results (Table 3). Under laboratory conditions, *M. brevifolia, M. maximus* and *R. preissii* displayed very strong physiological dormancy. When treated with plant signaling chemical combinations only *M. maximus* and *R. preissii* carried through chemical priming benefits to field emergence. *A. bunburyana* and *M. brevifolia* failed to emerge over the trial duration.

Discussion

The use of plant signaling chemical combinations can improve the germination and emergence of perennial pasture species under laboratory and glasshouse conditions. However, there is a general lack of translation of this benefit to field emergence, except in species with significant physiological dormancy issues associated with the seed at the time of treatment. These results suggest that whilst the use of plant signaling chemicals are useful in improving germination percentage, rate and germination tolerance to moisture stress, there needs to be a greater understanding of the biological and environmental factors that reduce this benefit under field conditions.

The efficacy of plant signaling chemicals in glasshouse and field conditions could be dictated by several factors: chemical dependencies, dormancy status of seed and stability of chemicals to temperature.

The use of kinetin may be an example of a chemical dependency. Here the efficacy of salicylic acid and kinetin to improve germination under moisture stress in laboratory conditions was a common theme across species. However, the use of kinetin in combination did not always work. Kinetin, a cytokinin, induces cell division but requires auxin to be present in order for it to be effective and the ratio of auxin to cytokinin is crucial during cell division (Mok and Mok, 1994). Auxins in plant tissues promote the production of ethylene, which along with GA3, interact with how seeds utilise light as a cue for germination. It is noteworthy that *A. semibacatta* and *R. preissii* did not require the presence of kinetin to benefit from priming and that both species have strong requirements for light in order to germinate. The efficacy of kinetin in species that did not respond significantly to the chemical may be due to a lack of auxin in the seed of these species.

The efficacy of chemicals on germination and emergence benefits can be affected by the dormancy status of seeds at the time of treatment. For example, the efficacy of GA3 as a plant signaling chemical, particularly in a number of the chenopod species tested, appears dependant on the after-ripening status (Hilhorst and Karssen, 1992). GA’s are actually not responsible for breaking down dormancy but combined with after-ripening relief, the inclusion of GA’s aid in the development of a GA-responsive system in seeds. GA biosynthesis in seeds, which occurs during seed imbibition, and dormancy function independently (Karssen and Lacka, 1986). Mangles Kangaroo paw (*Anigozanthos mangleisii*) shows a similar reaction with smoke water. Seeds become more smoke responsive with dry after-ripening (D. Merritt, *pers. comm*.).

Only two species in these experiments were regarded to be potentially in a state of after-ripening – *M. maximus*, whose seed was three months old, and *R. preissii* with four month-old seed. Both of these species responded well to PSCCs, including field conditions. Low germination in the laboratory of untreated seed show both species to be highly dormant (likely to be primary dormancy related), but highly responsive to ethylene for *M. maximus* and GA3 for *R. preissii*, respectively. For *M. maximus*, it could be that ethylene acts independently to after-ripening status. This conclusion may have substantial basis, as ethylene breaks two month old seed much better than GA3, yet in seven month old seed, the reverse occurs (C. Loo unpublished data). These observations need further investigation.

The stability of novel plant signaling chemicals, such as GA3, kinetin, salicylic acid, smoke water, ethylene and karrikinolide, under field conditions has not been studied. Most of these chemicals appear remarkably stable with >100^{o}C required for chemical decomposition (O'Neil, 2001) but there is no knowledge on karrikinolide and smoke water in this respect. Ethylene, on the other hand, is exceptionally volatile (O'Neil 2001). For successful use of ethylene on *M. maximus* under field conditions it appears that incorporation of chemical stability into the seed through hydro-priming will work much better than an external coating product on the seed, which is liable to become diluted and even removed altogether. Similarly, improved emergence with the use of smoke water in *D. eriantha* shows that this compound is stable and effective in the field. Similar field benefits in emergence from the use of smoke water have been observed in *Stylidium affine* (S. Turner, unpublished data).

The potential to observe chemical hydro-priming benefits on seed germination and emergence is dependent on sampling frequency, experimental duration and optimal temperatures for germination and emergence. For many non-dormant species, the priming benefit is largely due to increases in the rate of germination and emergence. Hence, benefits from these chemicals occur within the first few days after imbibition and differences between priming and control treatments diminish with time.

Both *A. bunburyana* and *M. brevifolia* failed to emerge throughout the course of the field trial. Both species performed poorly under glasshouse conditions as well, with low emergence rates compared with germination percentages in laboratory controls. Previous trials of both species in irrigated plots at South Perth also performed poorly. In that trial however, considerable mortality (23%) in *M. brevifolia* occurred in the first four weeks while no emergence was recorded in the same species at Shenton Park. This indicates that high moisture contents are required for germination of *M. brevifolia* in sandy soils.

These experiments suggest that potential field application of chemical hydro-priming appears limited to species which display highly dormant seed at the time of treatment, notably in *R. preissii, M. maximus, D. eriantha* and *A. saligna*. While this appears to benefit emergence in such species, carryover to plant establishment and first year survival remains unresolved. The use of this enabling technology displays great potential but the lack of translation to the field environment suggests that there are still many variables relating to priming that need to be understood. Future research should focus on developing an understanding of seed and chemical integrity after priming and how this relates to the seedbed environment.

Table 1. Identification of optimal combined plant signalling chemicals using moisture stress to differentiate control (no treatment) and priming treatments. Degree of significance between control and priming treatments are denoted by asterisks where * = *P* <0.05, **= *P* <0.01 and * = *P* <0.001. Standard errors are in parenthesis.**

Species	Identified combined plant signalling chemicals			
	Best option	% Final germ. (14 days) at CMS[a]		
		Control	PSCC[b]	P
A. semibaccata	GA3+SA	0 (0.0)	20 (2.3)	***
A. bunburyana	KAR1+SA	6 (1.3)	24 (3.3)	***
M. brevifolia	GA3+SA+K	2 (0.4)	11 (2.2)	**
P. ciliata	Ethy+SA+K	13 (3.1)	19 (4.2)	ns
T. ponticum	SW+SA	11 (2.4)	14 (2.8)	ns
R. preissii	GA3+SA	4 (1.9)	29 (4.9)	***
M. sativa	SW+SA	21 (3.8)	27 (4.4)	ns
M. maximus	Ethy+SA	3 (0.4)	16 (3.3)	**
P. clandestinum	Ethy+SA+K	16 (3.6)	19 (4.9)	ns
C. gayana	SW+SA+K	23 (4.4)	28 (3.9)	ns
S. sphacelata	KAR1+SA+K	7 (1.0)	16 (2.2)	**
D. eriantha	SW+SA	11 (0.7)	15 (1.1)	ns
L. bainesii	-	-	-	
M. atropurpureum	KAR1	26 (2.5)	39 (4.3)	*
A. saligna	KNO3	27 (3.0)	33 (5.5)	ns

[a]Critical moisture stress, defined as the water potential that retards germination by 75% of maximum germination

[b]Plant signaling chemical combination treatment

Table 2. **Percent germination/emergence in the laboratory (14 days), glasshouse (30 days) or field (30 days) in seeds un-treated, water primed (H_2O) or primed with combined plant signalling chemicals (PSCC). Standard error is denoted in parenthesis. Within each environment, PSCC treatments within a species that are significantly different (*P* <0.05) compared to controls, are denoted by an asterisk (*)**

Species	Laboratory (26/13°C)			Glasshouse (27/15°C)			Field		
	Control	H_2O	PSCC	Control	H_2O	PSCC	Control	H_2O	PSCC
A. semibaccata	32 (4.0)	53 (9.6)	100 (0.0)	24 (3.3)	30 (3.9)	46 (4.1)*			
A. bunburyana	12 (3.0)	27 (4.9)	39 (5.5)*	8 (1.0)	6 (1.3)	10 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)
M. brevifolia	8(0.0)	5 (2.7)	37 (5.3)*	4 (0.8)	5 (1.1)	12 (2.4)*	0 (0.0)	0 (0.0)	0 (0.0)
P. ciliata	81 (1.5)	77 (5.0)	92 (4.6)*	72 (6.8)	78 (11.2)	75 (4.2)	0 (0.0)	1 (0.7)	1 (0.7)
T. ponticum	99 (1.3)	100 (0.0)	100 (0.0)	96 (2.1)	98 (1.1)	97 (2.6)	55 (3.2)	53 (3.0)	52 (10.0)
R. preissii	16 (4.6)	24 (8.0)	95 (3.5)*	22 (2.0)	28 (3.3)	48 (5.1)*	1 (1.0)	6 (3.2)	17 (3.5)*
M. sativa	100 (0.0)	100 (0.0)	100 (0.0)	88 (6.7)	84 (4.4)	92 (7.2)	14 (2.6)	19 (2.5)	25 (3.9)
M. maximus	9 (1.0)	45 (3.8)	83 (2.3)*	11 (1.3)	34 (2.9)	56 (2.2)*	11(1.3)	34 (2.9)	56 (2.2)*
P. clandestinum	89 (1.7)	89 (2.3)	95 (2.6)	23 (5.6)	48 (2.7)	45 (3.8)*	38 (7.2)	36 (1.0)	40 (4.2)
C. gayana	20 (3.0)	28 (7.5)	73 (8.6)	35 (6.5)	51 (1.7)	46 (10.1)	64 (4.5)	51 (10.7)	67 (9.8)
S. sphacelata	53 (4.7)	41 (4.3)	79 (4.6)*	28 (2.9)	33 (5.6)	40 (3.4)*	26 (4.3)	36 (7.9)	36 (8.2)
D. eriantha	79 (1.3)	76 (5.4)	100 (0.0)*	30 (4.6)	50 (6.3)	71 (6.5)*	31 (4.1)	52 (4.6)	47 (7.3)*

L. bainesii	33 (5.0)	42 (6.0)	-	5 (2.2)	6 (1.2)	-	0.5 (0.3)	1 (0.8)	-
M. atropurpureum	44 (4.6)	54 (3.8)	56 (5.9)	19 (1.3)	21 (1.3)	30 (3.3)*	30 (3.7)	27 (1.8)	31 (1.7)
A. saligna	64 (4.7)	71 (8.6)	94 (6.5)*	7 (1.8)	8 (2.2)	19 (1.7)*	14 (2.6)	19 (2.5)	25 (3.9)

Direct seeding of old man saltbush (*Atriplex nummularia*)

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Introduction

Saltland is a difficult environment for plant establishment and growth. Our understanding of the germination and establishment processes in these hostile soils is rudimentary. Farmers seeking to establish halophytic shrubs on saltland can either: (a) seed directly using the niche seeding technique developed by C.V. Malcolm and colleagues in the 1970s (Malcolm and Allen, 1981), which deposits *Atriplex* fruits mixed with a vermiculite mulch at 1-3 m intervals along a raised M-shaped bank; or (b) they can use commercial tree planters to plant nursery-raised seedlings. The use of nursery-raised seedlings is generally much more reliable than niche seeding (Barrett-Lennard *et al*. 1991). However, direct seeding (~$100–150/ha) is far cheaper than the planting of nursery-raised seedlings (~$450/ha). Farmers, therefore, face a trade off between risk and price: the establishment technique that is most reliable is too expensive to implement; the accessibly priced technique is too risky.

Furthermore, the cost of sowing these species for rehabilitation of large pastoral zone areas is currently prohibitive and too risky. If prices for *demonstrably reliable* saltland and rangeland pasture establishment can be brought down to ~$120/ha, there would be substantial farmer and grazier adoption. Furthermore, there is a high chance that the developed techniques would be appropriate to the adoption of direct seeding for applications in the rangeland and arid areas of the wheatbelt.

In a pilot project, with SGSL and Salinity CRC support, DAWA and Kings Park have identified several seed treatments in the laboratory with a significant impact on germination. These relate to bract removal, light treatment and chemical stimulation. Most work has been conducted on the three major saltbush (*Atriplex*) species sown commercially– river saltbush (*A. amnicola*), old man saltbush (*A. nummularia*) and wavy leaf saltbush (*A. undulata*). Some work has also been conducted with creeping saltbush (*A. semibaccata*) and silver saltbush (*A. bunburyana*). These treatments are now ready for field validation, with the most promising options to move into 'commercialisation'.

Engineering the sowing niche is another means for improving halophyte establishment. Maximum growth of shrubs is possible with stands of ~1,000 stems per hectare (Malcolm *et al*. 1988; Barrett-Lennard 1993). Thus, an outlay of \$100–\$120 per ha for establishment equates to a cost of 10–12 cents per seed placement. This allows for the possibility of relatively expensive micro-engineering options to help overcome a number of stresses affecting establishment including salinity, waterlogging, inundation, drought, weeds, insects, grazing, and low temperatures/frost (Malcolm, 1972; Jennings *et al.* 1993; Barrett-Lennard *et al*. 2003). These will be initially investigated on a small scale and then on a larger commercial scale in saline and rangeland environments. Links will

Previous experiments had shown that old man saltbush could be successfully established from seeds using conventional seeding equipment. Germination was also found to be stimulated by priming seeds with chemical signalling compounds, such as kinetin. Glasshouse experiments had shown differences in ability to establish from seeds between subspecies of *A. nummularia*, with ssp. *spathulata* being more difficult to establish from seed than ssp. *nummularia*. Genotype differences for ability to establish were also observed within ssp. *nummularia*.

Methods

Two trials were sown in 2009 to confirm previous findings in two different environments and two different soil types. The following hypotheses were tested:

1. Old man saltbush can be successfully established from seed using conventional seeding machinery;
2. The use of priming with the chemical signalling compound, kinetin, enhances germination in the field;

3. Subspecies *nummularia* can be more readily established from seed than ssp. *spathulata*;
4. Genotypes of ssp. *nummularia* differ in their ability to establish from seed.

Treatments consisted of four genotypes, each with and without priming with 0.05 mM kinetin. Three genotypes were of ssp. *nummularia*, with the fourth being a commercial seed source of ssp. *spathulata*. Two genotypes of ssp. *nummularia* were obtained by Ron Yates from the property of Michael Lloyd at Pingaring. One of these (called 'Emergent type') was obtained from a bush that was observed to have a high density of seedling recruits surrounding it, which was subsequently found to have high establishment rates in the glasshouse. The other ('Non-emergent type') was from a bush with no seedling recruits, which was found to have low establishment rates in the glasshouse. The third ssp. *nummularia* genotype was a commercial source of cv. De Koch.

Seeds were screened and viability was checked using the Kings Park X-ray machine. Only viable seeds were used.

Trials were sown on a well-drained loamy soil at Mingenew (360 km N of Perth) and on a saline, waterlogged, sandy loam at Wagin (250 km SE of Perth). Weed control consisted of a double knockdown with glyphosate (July 17 and August 19 at Mingenew and August 3 and August 31 at Wagin).

Plots consisted of 8 m single rows, into which 200 seeds of each treatment were sown unto uncultivated soil using an experimental cone seeder. Treatments were replicated four times in a randomised block design. Seeds were sown 5-10 mm below the surface into 3 cm deep furrows and compacted into the soil surface with press wheels. Sowing dates were August 27 at Mingenew and September 7 at Wagin. The Mingenew site was sprayed with Talstar and Lorsban on September 3, while the Wagin site was sprayed with Talstar on September 14.

Measurements

Seedling counts were conducted at Mingenew on September 29 (34 days after sowing) and at Wagin on October 7 (30 days after sowing). Analyses of Variance were conducted on treatment means to determine whether establishment density differed between genotypes and priming treatments.

Results

Seedling counts per metre of row are shown for Wagin in Table 1 and for Mingenew in Table 2. Establishment was much higher at Wagin. Significant differences occurred between saltbush genotypes at both sites, with the Emergent type having significantly higher plant numbers than the Non-emergent type. Subspecies *spathulata* also had very low establishment at both sites. There were no significant effects of priming nor of the interaction between genotype and priming at either site. Photographs of the Wagin site is shown in Figure 1 and of Mingenew is shown in Figure 2.

Discussion

These results confirm that old man saltbush can be established by direct seeding. Precise sowing depth of 5-10 mm appears to be critical for success. Use of kinetin as a seed priming agent appears to be less important.

These trials confirmed the unsuitability of ssp. *spathulata* for direct sowing. In fact, there appears to be a lack of awareness of differences between subspecies in the saltbush nursery and seed trade. Other work in the Enrich project has demonstrated much greater palatability of ssp. *nummularia* than ssp. *spathulata*. A greater distinction needs to be made between these subspecies, in order that the public buys the type that will be best suited to their needs.

These results confirm previous glasshouse findings of genotypic differences within ssp. *nummularia* for ability to establish from seed. Seed of the Emergent and Non-emergent types have been forwarded to the Future Farm Industries CRC saltbush breeder, for inclusion of the ability to establish from seed as a breeding objective.

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Acknowledgements

This work was conducted as part of the project “Reliable establishment of non-traditional perennial pasture species”, with funding provided by the Future Farm Industries CRC, Meat & Livestock Australia, Australian Wool Innovation and Land and Water Australia. The support of the Saltland Pastures Association is also acknowledged. We are grateful to Chris English, Justin Bagley and their families for use of their properties.

Table 1. Seedlings per metre of old man saltbush genotypes with and without seed priming with 0.05 mM kinetin at Wagin

Genotype	Primed	Un-primed	Mean
de Koch	0.91	1.19	1.05
Emergent type	2.34	4.19	0.37
Non-emergent type	2.25	1.66	1.95
ssp. *spathulata*	0.00	0.16	0.08
Mean	1.38	1.80	1.59
Genotype difference	P<0.001		
l.s.d.	1.217		
Priming difference	Not significant		
Genotype x priming differences	Not significant		

Table 2. Seedlings per metre of old man saltbush genotypes with and without seed priming with 0.05 mM kinetin at Mingenew

Genotype	Primed	Un-primed	Mean
de Koch	0.47	0.09	0.28
Emergent type	1.22	0.91	1.06
Non-emergent type	0.16	0.03	0.09
ssp. *spathulata*	0.03	0.13	0.08
Mean	0.47	0.29	0.38
Genotype difference	$P<0.001$		
l.s.d. ($P = 0.05$)	0.474		
Priming difference	Not significant		
Genotype x priming differences	Not significant		